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THE EFFECT OF THE CONCENTRATION OF THE CULTURE SOLUTION ON VEGETABLES GROWN IN SAND

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ONE main purpose of previous similar experiments was to determine the effects of varying the supply of an element on the appearance and growth of vegetables. The results of variation in the total concentration of the important elements in the culture solution, the ratios of these elements to each other being kept constant, are investigated here. These experiments constitute an effort to determine for a given vegetable and given sources of the nutrients, the optimum concentration of the soil (sand) solution.

EXPERIMENTAL

Two control solutions were used, one for lettuce (C, Lettuce, Table 1), and the other for the root crops (C, Root crops, Table 1). These two solutions were known to suit the crops with which they were used, and they were prepared from salts that have been described (Woodman, 1939). Solutions A, B, D, E, and F, Table 1, for both lettuce and root crops, contained respectively $\times 3$, $\times 2$, $\times \frac{1}{2}$, $\times \frac{1}{3}$ and $\times \frac{1}{4}$ the quantities of the elements present in the two control ones. As the ferrous sulphate that contained the iron was constant in amount in all solutions, the quantities of sulphur did not quite vary as $A:B:C:D:E:F = 3:2:1:0{\cdot}5:0{\cdot}33:0{\cdot}25$. Minor elements were included in the solutions (subscript to Table 1). The small quantities of the combined elements (mainly sulphur as sulphate) necessarily introduced by the inclusion of salts containing the minor elements were ignored when compiling Table 1.

TABLE 1. *Concentration in p.p.m. of important elements in the culture solutions*

Solution ...	N	P	K	Ca	Mg	Fe	S	Na
Lettuce:								
A	98·88	65·52	67·32	27·09	15·15	1·01	48·16	259·68
B	65·92	43·68	44·88	18·06	10·10	1·01	32·30	173·12
C	32·96	21·84	22·44	9·03	5·05	1·01	16·44	86·56
D	16·48	10·92	11·22	4·52	2·53	1·01	8·51	43·28
E	10·99	7·28	7·48	3·01	1·68	1·01	5·87	28·85
F	8·24	5·46	5·61	2·26	1·26	1·01	4·54	21·64
Root crops:								
A	98·88	24·57	67·32	40·65	7·59	1·01	38·17	198·93
B	65·92	16·38	44·88	27·10	5·06	1·01	25·64	132·62
C	32·96	8·19	22·44	13·55	2·53	1·01	13·11	66·31
D	16·48	4·10	11·22	6·78	1·27	1·01	6·85	33·16
E	10·99	2·73	7·48	4·52	0·84	1·01	4·76	22·10
F	8·24	2·05	5·61	3·39	0·63	1·01	3·71	16·58

All the above solutions contained 0·0681 p.p.m. of boron as borax, 0·1847 p.p.m. of manganese as the sulphate (the tetrahydrate was used), 0·0682 p.p.m. of zinc as the sulphate, 0·0095 p.p.m. of aluminium as ammonium alum, 0·0164 p.p.m. of the ammonium radicle, $NH_4^{\cdot}$, partly as ammonium alum, and partly as ammonium sulphate, and 0·0153 p.p.m. of copper as the sulphate. The salts were *AnalaR*.

Plant pots were used for culture jars, as the effect of the large variations in concentration of the solutions used should have swamped any possible influences due to retention or supply of elements by such pots. Moreover, as a precaution, the pots were boiled for 20 min. in paraffin wax previous to the experiment. Each pot contained 13 lb. of a sand (Woodman, 1939) that was retained in the pot by a pad of glass-wool placed over the outlet of the pot and weighted down by a piece of waxed pot. The pots were arranged in a greenhouse as a system of randomized blocks. There were six treatments for

every crop, with solutions A–F. As there were sixteen replications for the radishes, eighteen each for the carrots, lettuces, and turnips, and twenty for the onions, the total numbers of cultures in each of the five experiments were: radishes, 96; carrots, lettuces, and turnips, 108 each; and onions, 120.

The sand in a pot was wetted by the appropriate culture solution. The seed was now sown in the pots, and the resulting seedlings were thinned to leave one per pot. Every pot received a definite volume of the appropriate solution at specified intervals.

The numerical data obtained

The eleven values obtained from each culture have been discussed previously, and a full description of the tables of summaries of results, together with methods of interpretation, have been given (Woodman, 1940). The data for the five crops are contained in Table 2. The roots of root crops were arbitrarily taken, as in horticultural practice, to be the edible portion plus the associated fibrous roots.

Table 2. *Summaries of results. Weights in g.*

Description of data		Treatment mean for						Mean of all results	S.E.
		A	B	C	D	E	F		
				Lettuce: May King					
Tops, FW	SSS	92·64	101·7	73·53	32·03	20·61	14·30	55·80	6·686
		A=B>D=E=F; B>C; A=C>D=E=F							
Roots, FW	SSS	12·20	18·27	37·97	23·45	15·57	10·33	19·63	1·615
		C>D>B>A=F; D>B=E>F; B=E; A=E							
Whole plants, FW	SSS	104·8	120·0	111·5	55·48	36·18	24·63	75·43	7·902
		A=B=C>D=E; D>F; E=F							
Tops, DW	SSS	5·782	6·445	6·446	3·236	2·215	1·671	4·299	0·3911
		A=B=C>D=E; D>F; E=F							
Roots, DW	SSS	0·671	1·456	4·119	2·628	1·896	1·271	2·007	0·2407
		C>D>B=E>A; D>F; B=E=F; A=F							
Whole plants, DW	SSS	6·452	7·901	10·57	5·864	4·111	2·942	6·307	0·5439
		C>B>D>E=F; C>A>E=F; A=B; A=D							
Top/root, FW	SSS	7·80	6·67	2·00	1·39	1·35	1·41	3·44	0·3862
		A=B>C=D=E=F							
Top/root, DW	SSS	8·59	7·12	1·85	1·26	1·22	1·35	3·56	0·4307
		A>B>C=D=E=F							
Tops, % moisture	SSS	93·08	93·50	91·19	89·81	89·13	88·27	90·83	0·2988
		A=B>C>D=E; D>F; E=F							
Roots, % moisture	SSS	93·69	92·87	89·51	88·80	87·82	87·74	90·07	0·4833
		A=B>C>E=F; C=D; D=E=F							
Whole plants, % moisture	SSS	93·23	93·37	90·59	89·41	88·62	88·07	90·55	0·2458
		A=B>C>D>E=F							
				Carrot: Primo					
Tops, FW	SSS	4·98	6·74	9·09	6·59	4·91	3·53	5·97	0·7605
		C>A=B=D=E; B=D>F; A=E=F							
Roots, FW	SSS	12·43	14·78	22·08	21·30	17·50	12·94	16·84	1·807
		C=D>A=B=F; C=D=E; A=B=E=F							
Whole plants, FW	SSS	17·41	21·52	31·16	27·89	22·41	16·47	22·81	2·604
		C>A=B=E=F; C=D>A=F; B=D=E							

TABLE 2 (*continued*)

Description of data		A	B	C	D	E	F	Mean of all results	S.E.
		Treatment mean for							
				Carrot: Primo (*continued*)					
Tops, DW	SSS	0·668	0·917	1·212	0·917	0·716	0·517	0·825	0·09301
		C>A=B=D=E; B=D>F; A=E=F							
Roots, DW	SS	0·940	1·224	1·695	1·800	1·489	1·148	1·383	0·1640
		C=D>A=F; D>A=B=F; C=D=E>A; B=C=E							
Whole plants, DW	SS	1·61	2·14	2·91	2·72	2·21	1·67	2·21	0·2533
		C>A=B=F; C=D>A=F; A=B=E=F; B=D=E							
Top/root, FW	SSS	0·654	0·536	0·485	0·318	0·290	0·297	0·430	0·06955
		A=B>D=E=F; A=B=C; C=D=E=F							
Top/root, DW	SSS	1·071	0·937	0·817	0·527	0·498	0·487	0·723	0·1014
		A=B>D=E=F; A=B=C>E=F; C=D							
Tops, % moisture	NS	85·17	86·55	86·48	85·69	85·98	85·33	85·87	0·8039
Roots, % moisture	NS	91·00	92·20	92·23	91·53	91·50	91·12	91·60	0·6311
Whole plants, % moisture	NS	89·01	90·32	90·56	90·22	90·15	89·89	90·03	0·6979
				Onion: Unwin's Reliance					
Tops, FW	SSS	10·70	17·05	10·41	2·871	1·628	1·598	7·38	2·375
		A=B=C>D=E=F							
Roots, FW	SSS	21·30	21·86	40·71	30·11	17·68	18·44	25·02	4·354
		C>A=B=E=F; C=D; A=B=D=E=F							
Whole plants, FW	SS	32·00	38·91	51·12	32·98	19·31	20·04	32·39	5·933
		C>A=D=E=F; B=C>E=F; A=B=D							
Tops, DW	SSS	1·416	1·928	1·702	0·697	0·339	0·335	1·070	0·2552
		B=C>D=E=F; A=B=C>E=F; A=D							
Roots, DW	SS	2·185	2·174	3·973	3·009	1·724	1·982	2·507	0·4528
		C>A=B=E=F; C=D; A=B=D=E=F							
Whole plants, DW	SSS	3·602	4·103	5·675	3·705	2·063	2·317	3·577	0·6422
		C>A=D=E=F; B=C>E; A=B=D=F							
Top/root, FW	SSS	1·699	1·662	0·378	0·120	0·088	0·098	0·674	0·1730
		A=B>C=D=E=F							
Top/root, DW	SSS	1·991	2·100	0·578	0·301	0·201	0·170	0·890	0·2263
		A=B>C=D=E=F							
Tops, % moisture	SS	80·59	81·98	77·22	65·21	70·63	76·19	75·30	3·122
		A=B>D=E; A=B=C=F; C=F>D; C=E=F							
Roots, % moisture	SSS	86·65	90·28	90·42	90·08	90·14	89·17	89·46	0·5608
		B=C=D=E=F>A							
Whole plants, % moisture	SSS	84·60	88·34	88·69	88·59	89·19	88·46	87·98	0·7415
		B=C=D=E=F>A							

TABLE 2 (*continued*)

Description of data		Treatment mean for A	B	C	D	E	F	Mean of all results	S.E.
		Radish: Turnip, Red, extra early, Short Top Forcing							
Tops, FW	SSS	2·68	2·81	3·65	4·31	3·22	2·75	3·24	0·2662
		D>A=B=E=F; C=D>A=B=F; C=E							
Roots, FW	S	4·26	4·82	6·04	5·78	5·38	4·42	5·12	0·4821
		C>A=F; C=D>A; B=C=D=E; A=B=E=F; B=D=E=F							
Whole plants, FW	SS	6·94	7·63	9·69	10·09	8·60	7·17	8·35	0·6334
		C=D>A=B=F; C=D=E; A=B=E=F							
Tops, DW	SSS	0·208	0·220	0·292	0·353	0·302	0·280	0·276	0·02164
		C=D=E>A=B; D>A=B=F; C=E=F							
Roots, DW	NS	0·224	0·241	0·286	0·293	0·285	0·236	0·261	0·02161
Whole plants, DW	SSS	0·432	0·461	0·578	0·646	0·587	0·516	0·537	0·03558
		C=D=E>A=B; D>A=B=F; C=E=F							
Top/root, FW	NS	0·724	0·610	0·681	0·892	0·629	0·758	0·716	0·09986
Top/root, DW	NS	1·032	0·938	1·110	1·372	1·110	1·351	1·152	0·1325
Tops, % moisture	S	92·16	92·21	91·96	91·70	90·41	89·65	91·35	0·2453
		A=B=C=D>E>F							
Roots, % moisture	NS	94·54	94·90	95·15	94·86	94·58	94·41	94·74	0·2457
Whole plants, % moisture	SSS	93·60	93·92	93·93	93·57	93·02	92·53	93·43	0·2034
		A=B=C=D>F; B=C>E=F; A=D=E							
		Turnip: Milan Strap-leaved, Purple Top							
Tops, FW	SSS	38·04	39·12	21·57	11·42	7·53	5·76	20·57	1·396
		A=B>C>D=E; D>F; E=F							
Roots, FW	SSS	83·25	129·2	105·9	64·58	41·98	28·18	75·52	4·719
		B>C>A>D>E=F							
Whole plants, FW	SSS	121·3	168·3	127·5	76·00	49·50	33·94	96·09	5·639
		B>A=C>D>E=F							
Tops, DW	SSS	2·54	2·87	1·82	1·06	0·718	0·618	1·604	0·1700
		A=B>C>D=E=F							
Roots, DW	SSS	6·01	8·90	8·07	5·04	3·34	2·43	5·63	0·3652
		B=C>A=D>E=F							
Whole plants, DW	SSS	8·55	11·77	9·89	6·09	4·06	3·05	7·23	0·4510
		B>A=C>D>E=F							
Top/root, FW	SSS	0·499	0·311	0·210	0·180	0·188	0·219	0·268	0·01800
		A>B>C=D=E=F							
Top/root, DW	SSS	0·474	0·330	0·236	0·211	0·222	0·261	0·289	0·01815
		A>B>C=D=E=F							
Tops, % moisture	SSS	93·35	92·64	91·44	90·75	90·45	89·26	91·32	0·1988
		A>B>C>D=E>F							
Roots, % moisture	SSS	92·96	93·15	92·36	92·15	91·98	91·12	92·29	0·1921
		A=B>C=D=E>F							
Whole plants, % moisture	SSS	93·08	93·05	92·23	91·95	91·75	90·83	92·15	0·1731
		A=B>C=D=E>F							

Cultural details and discussion of results

Lettuce

Variety, May King. Sown, 2 Feb. 1939; germinated, 15 Feb.; singled, 17 Feb.; harvested, 5 May 1939. Temp. 50–55° F. Each pot received 12 l. of its appropriate solution (Lettuce solutions, Table 1) in lots of 250 c.c. four times a week.

For about 3 weeks there was no perceptible difference between the treatments. Then plants with A became the lightest green, and those with B the next lightest. Later, however, the difference in shade between these treatments and the others was not so pronounced. Twelve with A and sixteen with B were hearted at harvest; but with both treatments there was a strong tendency to deep marginal scorch or rot of the outer leaves of the hearts, so that the lettuces were unmarketable. Although the tops with these treatments were among the largest ($A = B > D$; $B > C > D$; $A = C$), the lettuces were variable in size. Both treatments yielded good roots.

Solution C resulted in lettuces of normal green. All eighteen were hearted up. On three only were there any traces of the rot or scorch of the heart leaves noticed with A and B, and these traces did not affect the marketability. The size of the lettuces was quite good ($A = C > D = E = F$), and they were remarkably even. The roots were a good, even set, the largest of all ($C > D > B > A = F$; $D > B = E$), and one result of this was that the whole plant was equal in size to those with A and B ($C = A = B$). The dry matter of the tops was also equal to the best ($C = A = B$), while that for the roots was the largest ($C > D$, the next largest), as was the dry matter of the whole plant ($C > B$). The top/root ratios with C and diluter solutions were statistically equal, and much less than with A and B ($C = D = E = F < A = B$, for fresh material, and $C = D = E = F < B < A$, for the dried); concentrated solutions evidently tended to top growth. There was a definite decrease in the moisture content of tops, roots, and whole plants, in passing from the most concentrated solutions, A and B, to the most dilute, E and F.

After 3 weeks, with D, E, and F, there was a tendency to bronzing and purpling, and the tinted patches became more pronounced in passing from D to E. This tinting and the order of tinting were probably due to the small and decreasing amounts of phosphate present in D, E, and F, though a simultaneous reduction in nitrogen should also be remembered in this connexion (Woodman, 1939, 1940). None of D was hearted at harvest, though four showed signs. The lettuces were regular, but much smaller than with C ($D < C$); the roots were good, and the next largest to C ($C > D > B$). Solutions E and F led similarly to small, immature, and non-hearted lettuces ($D = E = F$), which were also unmarketable because of even greater tinting than that with D.

Solution C thus yielded the best lettuces as judged by size, appearance, and maturity. It is thus apparent that C represents, for this lettuce, the optimum concentration of the soil (sand) solution as regards nitrogen, phosphorus, and potassium. Thus solutions of similar concentration have proved most successful previously in various critical experiments with this lettuce (cf. Woodman, 1939, 1940).

Carrot

Variety, Primo. Sown, 27 Apr. 1939; germinated, 8 May; singled, 9 May; harvested, 14 July 1939. The greenhouse was heated till 23 May to 50–55° F.; for the remainder of the experiment the temperature varied from 50 to 80° F. Each pot received 11·25 l. of its appropriate solution (Root crops, Table 1) in lots of 250 c.c. four times per week.

Solutions A and B yielded plants of paler green than did less concentrated solutions, and some of the tops were chlorotic, or showed a sepia brown scorch. Cultures with C were also at first slightly paler green than those with D, E, and F, but, by the third week, these plants had nearly assumed the normal green colour.

At harvest, plants with A and B were still similar in appearance to the description given above. The roots were of good colour, but variable in size, those of chlorotic plants being small. C yielded good tops, nearly as dark as those with D, E, and F, and normal roots. D, E, and F, resulted in tops of good appearance, smaller as the solutions became more dilute; F gave a very even sample. The roots were of normal colour, and decreased in size in passing through D, E, and F, at the same time becoming definitely longer and more tapering in shape.

The largest tops were with C ($C>B$, the next largest); $C=D$ were the largest whole plants. For the roots, $C=D=E$, with a bias in favour of C (Table 2); roots with more concentrated solutions were relatively small ($A=B<C=D$). Solutions C and D were, in all respects, the best solutions for the growth of this carrot.

Top/root ratios for fresh and dry matters were larger with the more concentrated solutions, A, B, and C, yielding statistically identical results ($A=B=C$), as also did the most dilute ones ($D=E=F$). The differences between the moisture contents of the tops, roots, and whole plants, were not significant (NS) for the treatments (Table 2).

Onion

Variety, Unwin's Reliance. Sown, 27 Apr. 1939; germinated, 7 May; singled, 9 May; harvested, 7 Sept. 1939. The greenhouse was maintained at 50–55° F. till 23 May; thereafter the temperature ranged from 50 to 85° F. Each pot received 19·5 l. of its appropriate solution (Root crops, Table 1) in lots of 250 c.c. four times a week.

Solution A yielded an uneven set of onions. Four or five were quite good, and the tops were of normal green subsequent to the seedling stages, when all were chlorotic to such an extent that seven cultures never recovered, and finally died previous to the main harvest. Solution B gave similar results, but with less severe symptoms, and one plant only had to be harvested prematurely. C and D yielded plants of good appearance and size, of darker green. Healthy but smaller plants were also obtained with E and F.

At harvest, onions with C and D were the largest and statistically equal, $C=D$, although there was a bias in favour of C, whose yield was actually about 33 % greater, and which had larger tops ($C>D$). A, B, E, and F, yielded smaller onions than C ($A=B=E=F<C$); but, neglecting the seven of A that had to be prematurely harvested, the remaining thirteen (three of which were rotten) had an average weight of 32·68 g., which compared well with 40·71 g. with C, and 30·11 g. with D. In the initial stages of growth, when every plant with A was chlorotic, C and D appeared to give far superior cultures; so that it is evident that small onion plants were affected more by the concentrated solutions than large ones, and that a

plant that survived a concentrated solution in the seedling and post-seedling stages, had a good chance of yielding a large onion eventually.

Solutions A, B, and C, yielded tops which were statistically equal in weight, both fresh and dry ($A = B = C$), despite the tendency to severe chlorosis with A and B. As the fresh and dry roots with A, B, E, and F, were statistically equal and small ($A = B = E = F$), the relatively great weights of tops with A and B were reflected in the top/root ratios. Thus, for both of these ratios, $A = B > C = D = E = F$. The moisture contents for the roots and whole plants were lowest with A, but, as with the carrots, a large degree of insensitivity was shown in this regard to change in concentration with the diluter solutions ($A < B = C = D = E = F$).

Radish

Variety, Turnip, Red, extra early, Short Top Forcing. Sown, 24 Nov. 1938; germinated, 30 Nov.; singled, 1 Dec.; harvested, 2 Feb. 1939. Greenhouse maintained at 50–55° F. Each pot received 8·75 l. of its solution (Root crops, Table 1) in quantities of 250 c.c. three or four times a week.

Two weeks after germination, a chlorotic mottling caused cultures with A and B to be lighter green than the others. Solutions C, D, E, and F, all gave tops of uniform, darker green.

For the fresh roots at harvest, $B = C = D = E$; the results for the dried roots were not significant (NS). The radish thus appears to be remarkably insensitive to the concentration of sand (soil) solution. For the fresh tops, $C = D$, and $C = E$, although $D > E$, and for the dried tops, $C = D = E$, furthering this notion of insensitivity. There was, however, a strong general bias in favour of C and D as the best solutions, both from appearance and size.

The top/root ratios for both fresh and dry material were unaffected (NS) by changes in total concentration; the moisture contents of the roots were also not significant (NS), and, for the tops and whole plants, were insensitive to changes in concentration over the range A–D inclusive.

Turnip

Variety, Milan Strap-leaved, Purple Top. Sown, 2 Feb. 1939; germinated, 13 Feb.; singled, 16 Feb.; harvested, 25 Apr. 1939. Temp. 50–55° F. Each pot received 11 l. of its appropriate solution (Root crops, Table 1) in lots of 250 c.c. four times weekly.

There was a definite tendency to chlorosis in parts with solution A, and the old leaves tended to wilt, turn yellow, and die off. The chlorosis resulting from B was less severe than with A. The top/root ratios with the two solutions were relatively large, and, for both fresh and dry material, $A > B > C = D = E = F$. The tops with C, D, E, and F, were of a uniform, dark green, free from chlorotic mottling or patches, and the top/root ratios were obviously smaller than with A and B. The cultures with these more dilute solutions were uniform in size and appearance.

As judged by yield, B was obviously the best sand solution; thus, for the fresh roots, $B > C > A > D > E = F$, and, for the fresh tops, $A = B > C > D = E$. But as B tended to chlorotic plants, C, with the next best yield of fresh roots and the maximum yield of dry roots ($B = C > A$), could be placed as the optimum solution.

Summary

Experiments were carried out to discover the optimum concentration of the sand or soil solution for certain vegetables. Six solutions were used (A, B, C, D, E, and F, that contained important nutritional elements in the same ratios to each other, although the total concentration of these elements varied as A : B : C : D : E : F = 3 : 2 : 1 : 0·5 : 0·33 : 0·25). The best solutions for each of the vegetables grown, as judged by a statistical comparison of yields and by the general appearance and marketability, are given.

I thank Mr T. W. McKean and Mr J. N. Leonard for their help in these experiments.

REFERENCES

Woodman, R. M. (1939). Phosphate deficiency and yield tests on sand cultures of May King lettuce. *J. agric. Sci.* **29**, 229–49.

—— (1940). The nutrition of lettuces grown as sand cultures under glass. *Ann. appl. Biol.* **27**, 5–16.

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THE EFFECT UPON THE GROWTH OF PLANTS OF WATERING WITH SOLUTIONS OF PLANT-GROWTH SUBSTANCES AND OF SEED DRESSINGS CONTAINING THESE MATERIALS

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Templeman (1939) described experiments carried out at Jealott's Hill during 1936 and 1937 to determine the effect upon the total growth of plants of the application of plant-growth substances. These studies were continued and extended and are recorded in this communication.

Mitchell (1938) reported that the growth of bean plants was repressed when they were sprayed with solutions containing 300 mg./l. of β-indolylacetic acid, and that weaker solutions had no effect. Solutions containing 10–300 mg./l. had little effect on marigolds although marked epinasty resulted. Various amounts of indolylacetic acid dissolved in oil or water and sprayed on oat and bean seeds that were later sown in the field had no effect on their germination or fresh weight of tops at harvest. Indolylacetic acid had no appreciable effect upon the growth of corn, bean, oat or soy-bean plants that were grown in the field and sprayed with solutions containing this substance. The fresh weights of the tops of bean plants grown out of doors and irrigated with water containing 0·01 and 1 p.p.m indolylacetic acid were slightly greater than those of the control plants. The fresh weights of tops of marigolds irrigated with water containing 0·01 p.p.m. of indolylacetic acid were not affected but were suppressed when a solution containing 1 p.p.m. was used. β-Indolylacetic acid had no effect upon the time of flowering of any of the plants studied. Sayre (1939) showed that for tomatoes the largest early and total yields were obtained when the plants at transplanting received a pint of a solution containing 20 oz. of monammonium phosphate and 10 oz. of potassium nitrate in 50 gal. of water, but that the addition of 50 mg. of α-naphthylacetic acid to 50 gal. of this solution depressed the yield of fruit. Kaiser & Albaum (1939) studied the early root and shoot growth of two varieties of oats (*Avena sativa*) in relation to growth substances. Aqueous solutions of 0·01–5·0 mg./l. of β-indolylacetic acid showed the same maximum inhibition and the same time of maximum inhibition at low concentrations, but was later for the variety "Fulghum" than "Black Norway" at higher concentrations. Concentrations up to 50 mg./l. increased root growth similarly in the two varieties, but concentrations below 0·002 mg./l. were ineffective. Shoots of "Black Norway" grew rapidly when treated with concentrations between 0·002 and 2·0 mg./l.; shoots of "Fulghum" were unaffected.

Amlong & Naundorf (1939) claimed increased growth of various species of plants after treatment of the seed with solutions of growth substances, but Hwang & Pearse (1940) showed that treatment of seeds of oats (*A. sativa*) and broad bean (*Vicia faba*) with dilute solutions of indolylacetic acid before planting did not result in increased growth (dry weight) of seedlings, and that higher concentrations retarded growth. This is in agreement

with earlier findings for white mustard (Templeman, 1939). By treating tomato seeds with 0·0021–0·21 % solutions of β-indolylacetic acid, Herbst (1939) demonstrated a slight increase in growth at first, but later no differences between treated and control plants.

Various workers have also described the results of experiments in which plant growth substances have been applied as dry seed dressings to seeds prior to sowing. For example, McRostie *et al.* (1938) described increases in straw yields of winter wheat from the inclusion of small quantities of growth substances in the organic mercurial seed dressing applied to the seed; these authors state that "the effects on grain yield are not as clear cut owing to the differential response of varieties to the same treatment". Assessment from their experiment of the real effects of growth substance treatment are impossible since the trial was unbalanced (the complete series of growth substance treatments was not applied to a single wheat variety) and apparently contained no real control, i.e. seed untreated with mercurial dressing. Stier & du Buy (1938) reported increases in growth of tomatoes in sandy soil as a result of treatment of seed and plants with growth substances but here again, as the authors themselves state, the replication and randomization of the plots in the field were not adequate to warrant broad generalizations and definite conclusions. Croxall & Ogilvie (1940) described increases in the yield of peas due to the incorporation of growth substances in the seed dressing, but since this paper contains results as percentages only with no actual values or statistical analyses, critical examination of the data included therein is not possible. Well-replicated experiments on this subject were conducted in 1938 by the Seed Propagation Division of the Eire Department of Agriculture (Anon. 1939), but no differences in germination, growth or yield of barley could be observed or measured.

In this paper full details of replication and statistical analysis are given for each experiment. Two series of experiments are described, (i) in which the growth substances were applied as aqueous solutions to plants growing in sand or soil, and (ii) application of the growth substance in the seed dressing prior to sowing the seed.

(i) Application of growth substances in solution

Sodium-α-naphthylacetate was the only substance used. The solid salt was dissolved directly in water and all solutions were used immediately after preparation.

Exp. 1: *Lettuce* (Table 1)

Lettuce seedlings were planted in boxes of soil (comprising 2 parts loam to 1 part silver sand) when 12 days old. There were nine plants in each sq. ft. box and four randomized blocks of eight boxes each. After 16 days, growth in the greenhouse, the boxes were treated with the following solutions of sodium-α-naphthylacetate (24 Jan. 1938):

(1) Control—distilled water.
(2) 1 : 30,000 sodium-α-naphthylacetate.
(3) 1 : 1,000,000 sodium-α-naphthylacetate.
(4) 1 : 2,500,000 sodium-α-naphthylacetate.
(5) 1 : 5,000,000 sodium-α-naphthylacetate.
(6) 1 : 10,000,000 sodium-α-naphthylacetate.
(7) 1 : 50,000,000 sodium-α-naphthylacetate.
(8) 1 : 100,000,000 sodium-α-naphthylacetate.

Treatments were given from then onwards at intervals of 1 or 2 days; 100–400 c.c. were applied at a time, according to the wetness of the soil. (All boxes received equal volumes at any one application.) Fifteen applications were made between 4 Jan. and 10 Mar. 1938, and the total volume applied was 4200 c.c. The weight of hormone applied was equivalent to 6098·4 g. down to 1·829 g./acre, according to the concentration of growth substance used. On 1 Feb., as the lettuces were pale in colour, each box was given 200 c.c. of a solution containing 40 g. ammonium sulphate in 6·4 l. of water.

No obvious differences were seen during the growth of the plants, although one or two plants died prior to harvest owing to damage by hydrogen cyanide fumigation. All plants were harvested on 11 and 15 Mar., two blocks on each of those days. The tops and roots were separated at soil level, the roots were washed with water, dried with blotting paper and weighed. Tops were obtained as soon as possible after cutting. Dry weights were obtained by drying the material in a steam oven for at least 4 hr. The dry weights of tops and roots per box were analysed statistically, and the figures are given as a percentage of the control mean in Table 1.

TABLE 1.* *Results of Exp.* 1

	1 (Control)	2	3	4	5	6	7	8	General mean	S.E.	Sig. diff.
Mean dry wt. of tops per box (g.)	100·0 (4·50)	45·0 —	90·3	107·5	106·1	112·2	111·4	107·4	97·5	7·75	22·8
Mean dry wt. of roots per box (g.)	100·0 (0·29)	117·9	96·6	106·8	111·1	109·4	106·8	111·1	107·5	11·34	33·4

* Throughout this paper figures in the tables are given as percentages of the control mean, except where stated or obviously otherwise. The actual control figure is given in brackets below the 100. All weights were obtained in g. and heights in cm. except where stated otherwise. Significant differences between treated and control plants are marked throughout with the appropriate sign, + or −. Where treatments as a whole are not significant, this is indicated by **. Where figures for individual treatments were so different from the range of the other treatments as to necessitate leaving them out of the statistical analyses, this is indicated by the same sign **.

Treatments were significant for tops only. The highest concentration of hormone (1 : 30,000) had a depressing effect on the growth of tops. There were no significant differences in root weight, but again the 1 : 30,000 solution had an effect in causing a difference in appearance of the roots, the tap root being thick and bearing many thick short laterals towards the top. The results show no stimulation of growth by any treatment.

Exp. 2: *Lettuce* (Table 2)

Solutions of sodium-α-naphthylacetate of the same concentrations as in Exp. 1, with the addition of a 1 : 100,000 solution (treatment 9) were watered (A) continually, (B) once only, on to lettuces growing singly in pots. Soil in the pots comprised 3 parts loam to 1 part silver sand to 1 part leaf mould, with 7 lb. of bone meal added to 4 cwt. of mixture. There were two sets of controls (treatments 1 and 10).

The pots were divided into two main blocks (A and B), each consisting of eight blocks of ten pots each, one for each treatment. The pots were randomized in each of the eight blocks. All pots were watered with Cheshunt compound on 28 Jan. and 2 Feb. to check damping off. The first hormone treatment for block A and the one and only hormone treatment for block B were applied on 3 Feb. 1938. 50 c.c. was given to each pot. Thereafter block B received only water, while block A received hormone solution in volumes of from 25 to 50 c.c. when required, receiving between 3 Feb. and 15 Mar. a total volume of 725 c.c. in thirteen applications per pot.

TABLE 2. *Results of Exp.* 2

	Controls 1 and 10	2	3	4	5	6	7	8	9	General mean	S.E.	Sig. diff. (*a*)	Sig. diff. (*b*)
A. Dry wt. of top per pot (g.)**	100·0 (0·159)	0·0** —	60·4 —	104·8	106·2	84·4	78·1	100·7	7·6** —	93·8	12·05	34·2	29·7
Dry wt. of root per pot (g.)**	100·0 (0·023)	0·0** —	82·3	138·5	149·6 +	134·1	99·1	119·9	13·3** —	117·7	18·50	52·6	45·5
B. Dry wt. of top per pot (g.)	100·0 (0·259)	13·5** —	91·1	84·8	79·4	108·5	92·2	109·4	50·9 —	90·7	10·28	29·0	25·1
Dry wt. of root per pot (g.)	100·0 (0·046)	16·2** —	96·2	97·8	90·7	115·7	85·2	105·2	47·5 —	93·1	12·80	36·1	31·3

(*a*) is for comparison of any treated, (*b*) is for comparison of Control *v.* any treated.

The plants were harvested on 16 and 17 Mar. The statistical analyses of the dry weights of tops and roots are summarized in Table 2. Treatments 2 and 9 caused considerable visible damage, especially in block A where they were applied continually. The plants tended to be small and the roots were found at harvest to be very small and brown. All plants treated with solution 2 in block A, four plants treated with 2 in block B and four plants treated with 9 in block A were killed. These treatments as well as treatment 7 in block A, which unaccountably caused the death of three plants, are omitted from statistical analyses.

Treatments A were not significant as a whole; but, when analysed separately, treatments A 3 and A 5 gave significant differences, 3 depressing the tops and 5 stimulating the roots. Treatment 9 in blocks B caused a significant depression in weight of tops and roots. Thus, sodium-α-naphthylacetate solutions applied in a number of doses at intervals to lettuce were toxic at 1 : 1,000,000, 1 : 100,000, and 1 : 30,000 of sodium-α-naphthylacetate, the toxicity increasing with the concentration. When applied once only, the solutions were less toxic and the 1 : 1,000,000 concentration did no damage. Root weight was increased slightly by continued application of 1 : 5,000,000 solution. There was no significant increases in dry weight of tops.

Exp. 3: *Lettuce* (Table 3)

A second experiment to compare the effect of a single application of hormone with that of several applications was carried out with lettuce. Plants sown 8 Jan. were potted into "Large 60" pots of soil of the same mixture as in Exp. 2. Treatments were as follows:

10 applications of sol. (50 c.c. per pot)	1 application of sol. (50 c.c. per pot)	mg. hormone per pot
(1) Control—distilled water	(6) Control—distilled water	0·0
(2) 1 : 100,000 hormone	(7) 1 : 10,000 hormone	5·0
(3) 1 : 1,000,000 hormone	(8) 1 : 100,000 hormone	0·5
(4) 1 : 10,000,000 hormone	(9) 1 : 1,000,000 hormone	0·05
(5) 1 : 100,000,000 hormone	(10) 1 : 10,000,000 hormone	0·005

There were five replicates and the treatments were randomized in blocks. Pots were given 50 c.c. of solution on 22 Feb. Treatments 2, 3, 4 and 5 were applied at intervals of 1 or 2 days, the tenth treatment being given on 14 Mar. The rest of the pots received only water after their single treatment.

Treatments 2 and 7 caused considerable damage. On 28 Feb. treatment 7 had caused severe wilting, treatment 2 was less severe, but the outer leaves were very chlorotic. Subsequently two of the plants in each case died, while the rest remained small and chlorotic. These treatments are omitted from the analyses given later. Treatments 3 and 8 caused chlorosis at first, but the plants had apparently recovered by 15 Mar., although their size was on the whole small compared with that of plants treated with other solutions. The plants were harvested on 22 Mar. (three replicates), and 23 Mar. (two replicates), fresh and dry weights for tops and roots being obtained. The statistical analyses of dry weights are summarized in Table 3.

TABLE 3. *Results of Exp.* 3

	Controls 1 and 6	2	3	4	5	7	8	9	10	General mean	S.E.	Sig. diff. (a)	Sig. diff. (b)
Dry wt. of top per pot (g.)**	100·0 (0·399)	19·3** —	92·5	101·3	108·3	8·0** —	76·7	119·8	100·0	99·8	12·54	36·3	31·5
Dry wt. of root per pot (g.)**	100·0 (0·065)	19·9** —	110·4	94·5	92·9	10·7** —	67·8	133·7	98·2	99·7	14·96	43·3	37·5

(*a*) is for comparison of any treated, (*b*) is for comparison of Control *v.* any treated.

Treatments as a whole were not significant. The only conclusion is that a single application of 1 : 10,000 sodium-α-naphthylacetate had a more rapid toxic action than applications of a solution ten times as dilute; but that by the time ten applications of the weaker solution had been made (and the weight of hormone per pot made up therefore to the same weight as in the single application of 1 : 10,000), the damage done was about equal for both treatments. There was no sign of stimulation by any of the treatments.

Exp. 4: *White mustard*

White mustard was sown on 11 Jan. 1938 in 1 sq. ft. boxes of builder's sand and watered with 50 c.c. of standard nutrient solution[1] per box every other day. When the seedlings had started to form the third leaf they were planted, twelve seedlings per box, in 1 sq. ft. boxes of soil comprising 3 parts loam to 1 part sand (9 Feb.). There were eight treatments and three replicates, and the boxes were randomized in blocks in the glasshouse. Treatments were as follows:

(1) Control—distilled water.
(2) 1 : 30,000 sodium-α-naphthylacetate.
(3) 1 : 1,000,000 sodium-α-naphthylacetate.
(4) 1 : 2,500,000 sodium-α-naphthylacetate.
(5) 1 : 5,000,000 sodium-α-naphthylacetate.
(6) 1 : 10,000,000 sodium-α-naphthylacetate.
(7) 1 : 50,000,000 sodium-α-naphthylacetate.
(8) 1 : 100,000,000 sodium-α-naphthylacetate.

400 c.c. were given at a time to each box. The first application was made on 11 Feb. when the mustard was about 4½ weeks old. Treatments were subsequently given whenever the soil required watering. Six applications were made between 11–28 Feb., 2400 c.c. of solution being applied altogether to each box. This was equivalent to a total application of from 3484 g. to 1·0454 g. hormone/acre. Plants treated with solution 2 became stunted compared with the controls. The plants were harvested on 3 Mar. Fresh and dry weights of tops and roots were obtained but examination of the figures showed a statistical analysis to be unnecessary, since the only difference due to treatment was that treatment 2 gave very low fresh and dry weights of tops. There was no stimulation.

Exp. 5: *White mustard* (Table 4)

This was similar to Exp. 4 except that sand instead of soil was the growth medium and that the toxic solution containing 1 : 30,000 parts of hormone was omitted from the treatments. The plants were sown in the boxes in which they were to be treated, so that no transplanting was necessary. Thirty-two 1 sq. ft. boxes of sand were sown with 20 mustard seeds each on 11 Mar. The boxes were randomized in 4 blocks with 8 boxes per block. The following eight treatments were given, the first application being made on 14 Mar. before signs of germination showed.

(1) 1 : 1,000,000 sodium-α-naphthylacetate.
(2) 1 : 2,500,000 sodium-α-naphthylacetate.
(3) 1 : 5,000,000 sodium-α-naphthylacetate.
(4) 1 : 10,000,000 sodium-α-naphthylacetate.
(5) 1 : 25,000,000 sodium-α-naphthylacetate.
(6) 1 : 50,000,000 sodium-α-naphthylacetate.
(7) 1 : 100,000,000 sodium-α-naphthylacetate.
(8) Control.

The treatment was continued at intervals of a few days, 300–400 c.c. being given at a time. As soon as the first leaf above the cotyledons had developed (29 Mar.) the hormone solutions were made up in standard[1] nutrient solution. On 4 Apr. the plants were thinned to 10 per box. Between 14 Mar. and 15 Apr. 14 applications of hormone, totalling 5500 c.c. per box, were made. The plants were harvested on 16 May (three replicates) and 17 May (one replicate). Fresh and dry weights of tops and roots were obtained. Fresh weights showed no significant differences. Dry weights of tops showed slight depression due to treatments 1, 3, 4 and 6, but not by 2, 5 and 7. These figures are summarized in Table 4. Dry weights for roots showed no significant differences.

TABLE 4. *Results of Exp.* 5

	1	2	3	4	5	6	7	8 Control	General mean	S.E.	Sig. diff.
Dry wt. of tops per box (g.)	80·5 —	98·6	86·3 —	86·0	95·9	85·7 —	98·0	100·0 (7·33)	91·4	4·47	13·2
Dry wt. of roots per box (g.)**	96·3	72·7	75·2	68·6	83·1	58·7	86·4	100·0 (6·05)	80·1	15·04	44·2

Exp. 6: *Tomato* (Tables 5, 6)

Tomatoes (variety Bill Hill Beauty), sown 6 Jan. 1938, were potted in "Small 60" pots of soil on 2 Feb. and transferred on Feb. 17 to "Large 60's" containing (*a*) soil (mixture 10 parts loam, 7 parts Sorbex-peat, 6 parts silver sand, and 1½ oz. superphosphate and 1 oz. of chalk per bushel) or (*b*) silver

[1] See Appendix.

sand. Treatments were as follows, the growth substance solution for sand pots being made up with standard nutrient solution:

(1) 1 : 100,000 sodium-α-naphthylacetate.
(2) 1 : 1,000,000 sodium-α-naphthylacetate.
(3) 1 : 5,000,000 sodium-α-naphthylacetate.
(4) 1 : 10,000,000 sodium-α-naphthylacetate.
(5) 1 : 25,000,000 sodium-α-naphthylacetate.
(6) 1 : 50,000,000 sodium-α-naphthylacetate.
(7) 1 : 100,000,000 sodium-α-naphthylacetate.
(8) 1 : 200,000,000 sodium-α-naphthylacetate.
(9) Control.
(10) Control.

There were ten replicates, five in sand and five in soil, for each treatment. Sand and soil pots were arranged on separate tables in the glasshouse in randomized blocks. The first application, of 100 c.c. per pot, was made on 17 Feb. This was too much for the sand pots and ran through slightly: subsequently only 50 or 25 c.c were applied at a time. On 10 Mar. it was obvious that solution 1 was stunting the growth of the tops. Observations on the size of the plants were made on 23 Mar. and are summarized in Table 5.

TABLE 5. *Exp.* 6: *plant size*

The number of plants for each treatment of a certain size is given on the appropriate line.

Observed size of plant	1	2	3	4	5	6	7	8	Controls 9	10
Soil:										
Large		1	2		2	3	3	2	4	3
Medium		1	3	3	2	2	2	3	1	2
Small	2	3		2	1					
Dead	3									
Sand:										
Large		5	5	5	5	5	5	5	4	3
Medium									1	2
Small	4									
Dead	1									

Treatment 1 is very toxic, especially in soil. The total volume of hormone solution received per pot between 17 Feb. and 28 Mar. 1938 was 750 c.c. The plants were harvested on 29 Mar. at the age of 3 months. Fresh and dry weights of tops and roots were obtained. Results are given in Table 6.

TABLE 6. *Results of Exp.* 6: *av. dry wt. of tomatoes per pot in g.*

	1**	2	3	4	5	6	7	8	Controls 9 and 10	General mean	S.E.	Sig. diff. (*a*)	(*b*)
Tops, soil**	0·9 —	22·0	49·2	29·1	76·0	98·5	77·6	68·4	100·0 (0·845)	69·0	24·47	69·9	60·6
Roots, soil**	5·0 —	27·3	37·3	38·5	69·6	105·6	63·4	69·6	100·0 (0·161)	67·9	21·92	62·6	54·3
Tops, sand**	9·8 —	106·8	108·4	129·0	131·2	104·0	187·6 +	141·0	100·0 (0·631)	123·1	24·75	70·7	61·3
Roots, sand**	16·0 —	173·3	139·0	141·2	203·2 +	117·6	172·2	158·3	100·0 (0·187)	145·0	29·44	84·1	72·9

(*a*) is for comparison of any treated, (*b*) is for comparison of Control *v.* any treated.

Treatments as a whole are not significant. However, separate tests of certain treatments against the control mean showed that treatment 7 had significantly stimulated tops in sand and that treatment 5 had significantly stimulated roots in sand. Treatment 2, although it appeared to have stimulated roots in sand, when tested separately against the control proved to be non-significant. Other concentrations had no significant effect.

(ii) APPLICATION OF GROWTH SUBSTANCE IN THE SEED-DRESSING PRIOR TO SOWING THE SEED

The growth substance was incorporated in the seed dressing which was talc, Agrosan, or Granosan.

Exp. 7: *Spring oats* (Table 7)

Spring Oats (Victory) were treated at the rate of 0·297% by weight with the following seed dressings:

(1) Agrosan G.
(2) Agrosan G containing 0·01% α-naphthylacetic acid.
(3) Agrosan G containing 0·10% α-naphthylacetic acid.
(4) Agrosan G containing 1·00% α-naphthylacetic acid.

Each lot was sown in the field in a strip of about 57 yd. long by 19 ft. wide, corresponding to two widths of the drill, and approximately 28 lb. of seed was sown in each strip (15 Mar. 1938). The experimentally treated strips were separated by similar strips sown with seed treated with Agrosan G. Between each strip was a path 1 ft. wide. There were no replicate strips.

Germination counts were made by throwing a yardstick at random and counting for 1 yd. along the row touched by the end of the stick: thirty counts were made for each of the strips, and for the separating control strips. Counts were made on three different days, and results indicated no differences between treated and control strips. Observations on the general growth of the strips again showed no differences. The field was somewhat patchy, owing to the previous presence of manure dumped in heaps, but this effect was similar for all strips.

A count of fertile tillers was made on 50 random yards for each strip, and two heights were measured at random in each of the yards. This was on 27 June when the plants had been growing for 15 weeks. These figures were analysed in pairs and tested for significance. The only fact that emerged from this was that the first of the control strips between the hedge and the treatment 1 strip (which was, of course, a control also) was low in both number of fertile tillers and in heights. This was probably due to position in the field. There were no significant differences between the other strips.

The field was harvested on 27 July. A representative strip, 30 yd. long, was taken from the centre of each experimental strip and yields of straw and grain were thus obtained for each strip. These figures are given in Table 7. It can be seen that, for both grain and straw, the seed dressed with hormonized dressings has not given higher yields than has the control seed.

TABLE 7. *Exp.* 7: *summary of growth data for oats treated with "hormonized" seed dressings*

	Control	1 0%	Control	2 0·01%	Control	3 0·10%	Control	4 1·00%
Germination count. 29 Mar. Total of 30 yd. counts	—	1239	1179	1273	1204	1306	1033	1150
Germination count. 1 Apr.	1715	2063	1802	1771	1910	1851	1904	1721
Germination count. 5 Apr.	1981	2123	1804	2066	1963	2036	1856	1888
Count of fertile tillers. 27 June	2175	3054	2757	3042	2890	2842	2731	2705
Height measurements. 27 June. Av. per plant, in cm.	136·90	155·26	158·90	156·50	154·42	143·38	147·64	147·32
Wt. of grain in lb.	—	96	99½	95	103½	96	97½	92
Wt. of straw in lb.	—	140	137½	123½	141½	114	125½	120

Exp. 8: *Spring barley* (Tables 8, 9)

Barley (variety Plumage Archer) was dressed at the rate of 0·223% by weight with the following seed dressings and planted in tall glazed pots containing soil or sand on 4 Apr. 1938.

(1) Control—not dressed.
(2) Control—Agrosan G.
(3) Agrosan G+0·01% α-naphthylacetic acid.
(4) Agrosan G+0·05% α-naphthylacetic acid.
(5) Agrosan G+0·10% α-naphthylacetic acid.
(6) Agrosan G+0·50% α-naphthylacetic acid.
(7) Agrosan G+1·00% α-naphthylacetic acid.
(8) Talc+0·01% α-naphthylacetic acid.
(9) Talc+0·05% α-naphthylacetic acid.
(10) Talc+0·10% α-naphthylacetic acid.
(11) Talc+0·50% α-naphthylacetic acid.
(12) Talc+1·00% α-naphthylacetic acid.

There were thus twelve treatments in soil and in sand, with five replicates in each, 120 pots being used altogether. The pots were randomized. The amount of α-naphthylacetic acid in p.p.m. of seed weight

was treatments 3 and 8, 0·223; treatments 4 and 9, 1·115; treatments 5 and 10, 2·23; treatments 6 and 11, 11·15; and treatments 7 and 12, 22·3.

The pots were kept equally damp as far as possible, and when the plants were 2 weeks old the sand pots were given standard[1] nutrient solution instead of water. On 4 May the plants were thinned to six per pot, and on 20 May to three, an average sample being left in each case. Germination counts, measurements of heights, and counts of tillers were made at intervals.

The plants were harvested at the age of 10 weeks, before ears had begun to form, since they were badly attacked by mildew. No difference could be detected in the degree of infestation of different treatments, and there was no difference between talc and Agrosan G seed-dressed pots. Tables 8 and 9 summarize the results for soil and sand cultures respectively. The figures given are the means for talc and Agrosan G dressings for each concentration of hormone, since talc *v.* Agrosan G was significant for the early tiller counts only. The only significant differences for the soil pots are in the rate index for germination, where the two highest concentrations of hormone gave a lowering of the rate. (See Appendix II for definition of rate index.)

TABLE 8. *Exp.* 8: *summary of results for soil pots. Talc and Agrosan G are averaged for each concentration of hormone*

	Control	Naphthylacetic acid 0·01 %	0·05 %	0·10 %	0·50 %	1·00 %	Mean	Sig. diff.
Rate index for germination	0·788	0·755	0·744	0·757 —	0·725 —	0·713 —	0·747	0·047
Heights, 25. iv. 38 (cm.)	9·87	10·06	10·02	9·84	9·51	9·72	9·84	0·38
Heights, 12. v. 38 (cm.)	132·3	133·1	133·8	132·1	127·5	131·5	131·7	4·8
Tillers, 12. v. 38	13·2	13·3	13·1	12·7	13·1	13·4	13·1	1·8
Heights, 31. v. 38 (cm.)**	13·4	135·6	135·2	136·2	136·7	139·1	136·7	5·8
Tillers, 31. v. 38**	17·3	17·3	16·6	17·5	17·4	19·2	17·6	2·6
Dry wt. of tops (g.)**	7·79	7·89	7·88	7·59	7·71	8·14	7·83	0·86
Dry wt. of roots (g.)**	4·52	4·58	4·10	4·18	3·98	4·55	4·32	1·49

TABLE 9. *Exp.* 8: *summary of results for sand pots. Talc and Agrosan G are averaged for each concentration of hormone*

	Control	Naphthylacetic acid 0·01 %	0·05 %	0·10 %	0·50 %	1·00 %	Mean	Sig. diff.
Rate index for germination	0·729	0·763	0·791	0·752	0·750	0·704	0·748	0·073
Heights, 25. iv. 38 (cm.)	7·60	7·77	7·71	7·43	7·26	7·17 —	7·49	0·41
Heights, 12. v. 38 (cm.)	118·2	123·1 +	118·5	121·3	115·4	115·4	118·7	4·4
Tillers, 12. v. 38	22·1	20·5 —	21·1	21·6	20·5 —	19·8 —	20·9	1·4
Heights, 31. v. 38 (cm.)**	139·1	142·7	139·8	141·7	138·0	139·6	140·2	3·9
Tillers, 31. v. 38**	35·7	33·9	34·7	35·8	36·5	34·5	35·2	4·1
Dry wt. of tops (g.)	14·19	13·59	12·99 —	14·42	13·35	13·43	13·66	0·96
Dry wt. of roots (g.)	7·84	8·30	7·43	8·95	7·40	7·69	7·94	2·26

In sand the rate index has no significant differences, but there are various differences in the early height measurements and tiller counts. 25 Apr., depression in height at 1·00 %: 12 May, stimulation in height at 0·01 %: depression in tiller count at 0·01, 0·5 and 1·00 %. Later height and tiller counts gave no significant differences. For the dry weights of tops in sand, the 0·05 % dressing gave a significantly low value. This is somewhat anomalous since neither lower nor higher concentrations had any effect.

In conclusion, it was found that seed dressings containing 1 or 0·5 % α-naphthylacetic acid in Agrosan G or talc, depressed the rate of germination of barley in soil, but had no later effects. In sand the rate of germination was unaffected, but the height of the plants was depressed by the 1 % dressing at first, and the tillering was depressed at first by 1 and 0·5 %. Later these effects were no

[1] See Appendix.

longer apparent. Dressings containing lower percentages of α-naphthylacetic acid had no effect: one suggestion of stimulation in height by 0·01 % dressing was offset by a simultaneous depression in tillering.

Exp. 9: *Sugar beet* (Table 10)

Sugar beet "seed" was dressed at the rate of 0·558 % with the same "hormonized" talcs and Agrosan G as used for barley in the previous experiment.

(1) Control—not dressed.
(2) Control—Agrosan G.
(3) Agrosan G + 0·01 % α-naphthylacetic acid.
(4) Agrosan G + 0·05 % α-naphthylacetic acid.
(5) Agrosan G + 0·10 % α-naphthylacetic acid.
(6) Agrosan G + 0·50 % α-naphthylacetic acid.
(7) Agrosan G + 1·00 % α-naphthylacetic acid.
(8) Talc + 0·01 % α-naphthylacetic acid.
(9) Talc + 0·05 % α-naphthylacetic acid.
(10) Talc + 0·10 % α-naphthylacetic acid.
(11) Talc + 0·50 % α-naphthylacetic acid.
(12) Talc + 1·00 % α-naphthylacetic acid.

The "seed" was sorted and sieved to a medium size. Seeds were planted, fifty seeds per pan, in sand or soil. Each treatment had four replicates in sand and four in soil. The pans were placed in an unheated frame and randomized in blocks. The seeds were planted on 26 Apr., and the plants were harvested on 17 June, fresh and dry weights of tops and roots being obtained. The plants were small when harvested, and the roots had hardly started to swell.

Neither germination counts nor weights of tops and roots showed any significant differences; i.e. there were no evidences for any stimulating effect of hormonized seed dressings (see Table 10).

Table 10. *Exp.* 9: *growth data for sugar beet treated with "hormonized" seed dressings. Figures averaged for talc and Agrosan G*

		Control	α-Naphthylacetic acid 0·01 %	0·05 %	0·10 %	0·50 %	1·00 %	Mean	Sig. diff.
Soil:	Rate index for germination**	0·488	0·430	0·391	0·426	0·449	0·415	0·433	0·090
	Final count for germination**	70·0	69·9	68·1	74·5	69·5	67·3	69·9	12·2
	Fresh wt. of tops (g.)**	93·6	100·4	95·6	84·9	88·8	81·1	90·7	37·6
	Dry wt. of tops (g.)**	7·10	6·84	6·46	6·21	6·46	6·11	6·53	2·02
	Fresh wt. of roots (g.)**	16·6	12·2	11·6	12·8	12·4	12·9	13·1	4·5
	Dry wt. of roots (g.)**	1·80	1·19	1·25	1·41	1·31	1·30	1·38	0·62
Sand:	Rate index for germination**	0·493	0·459	0·497	0·497	0·483	0·498	0·488	0·078
	Final count for germination**	68·0	66·1	68·5	71·0	68·5	72·6	69·1	6·9
	Fresh wt. of tops (g.)**	105·7	97·9	99·7	92·4	102·4	103·6	100·3	20·5
	Dry wt. of tops (g.)**	6·96	6·88	6·59	7·18	7·31	7·29	7·03	1·60
	Fresh wt. of roots (g.)**	20·7	21·9	19·5	19·0	21·8	20·3	20·5	4·2
	Dry wt. of roots (g.)**	2·05	1·85	1·89	1·93	2·55	1·80	2·01	0·78

Exp. 10: *Cereal seedlings* (Table 11)

To determine the effects of "hormonized" seed dressings on cereals at an early stage, seeds of oats, barley, and wheat (sorted to a medium size) were dressed at the appropriate rates (0·297, 0·223 and 0·208 % respectively) with talc dressings containing α-naphthylacetic acid at the following concentrations:

(1) Control—not dressed.
(2) Control—talc.
(3) 0·01 % α-naphthylacetic acid in talc.
(4) 0·05 % α-naphthylacetic acid in talc.
(5) 0·1 % α-naphthylacetic acid in talc.
(6) 0·5 % α-naphthylacetic acid in talc.
(7) 1·0 % α-naphthylacetic acid in talc.

They were then planted, sixteen seeds/1 sq.-ft. box, the boxes containing sand with 2 lb. C.C.F. fertilizer (finely ground) to 12 cwt. of sand. There were two boxes per treatment. The boxes were randomized in blocks. Germination counts were made, but the treatments did not reach significance for either final count or rate index (see Table 11). The plants were harvested when 3 weeks old. There was some variation between boxes, but no obvious variation between treatments. Fresh and dry weights of the plants were analysed, but here again no significant differences were found. It appeared, therefore, that there was no stimulation or other effect from the "hormonized" seed dressings, but it was felt that the experiment should be repeated using more replicates.

TABLE 11. *Results of Exp.* 10: *growth data for cereal seedlings treated with "hormonized" seed dressings*

	Control not dressed	α-Naphthylacetic acid in talc 0·0 %	0·01 %	0·05 %	0·1 %	0·5 %	1·0 %	General mean	S.E.	Sig. diff.
Wheat: Final count for germination**	100·0 (11·0)	104·5	100·0	136·4	118·2	109·1	100·0	109·7	16·97	58·7
Rate index for germination**	100·0 (0·479)	123·5	117·1	120·8	111·8	113·3	110·0	113·8	10·10	34·9
Fresh wt. per plant (g.)**	100·0 (0·346)	118·7	99·9	126·8	99·0	113·5	101·7	108·5	16·48	57·0
Dry wt. per plant (g.)**	100·0 (0·056)	112·5	101·8	133·9	95·5	104·5	94·6	106·1	12·78	44·2
Oats: Final count for germination**	100·0 (14·5)	93·1	103·4	106·9	100·0	100·0	103·4	101·0	6·52	22·5
Rate index for germination**	100·0 (0·846)	82·9	99·0	99·1	89·5	100·7	94·3	95·1	4·73	16·4
Fresh wt. per plant (g.)**	100·0 (0·526)	77·2	86·2	81·4	92·8	92·2	76·1	86·6	10·44	36·1
Dry wt. per plant (g.)**	100·0 (0·068)	82·2	92·6	85·9	82·2	93·3	81·5	88·3	8·31	28·8
Barley: Final count for germination**	100·0 (13·5)	85·2	107·4	81·5	107·4	92·6	103·7	96·8	11·85	41·0
Rate index for germination**	100·0 (0·495)	104·6	112·0	95·4	104·2	102·8	120·3	105·6	10·40	36·0
Fresh wt. per plant (g.)**	100·0 (0·334)	95·8	130·5	99·1	116·2	99·3	147·6	112·6	18·36	63·5
Dry wt. per plant (g.)**	100·0 (0·049)	97·9	111·3	109·3	113·4	99·0	122·7	107·7	13·78	47·7

Exp. 11: *Cereals* (Tables 12–18)

Accordingly, in this experiment the cereals were dressed with Granosan[1] containing β-indolylacetic acid or α-naphthylacetic acid. Treatments were as follows:

(1) Control—not dressed.
(2) Control—Granosan.
(3) 0·4 % naphthylacetic acid in Granosan.
(4) 0·4 % indolylacetic acid in Granosan.

The recommended rate of ½ oz./bushel was used. This was equivalent to (in terms of weight of dressing % of seed weight): 0·062 % for barley, 0·079 % for oats, and 0·050 % for wheat, and gives 2·48, 3·16 and 2·00 p.p.m. of hormone per seed weight, for barley, oats and wheat respectively. For each cereal there were five replicates in sand and five in soil, wooden boxes being used as before, and randomization was in blocks. The seeds were in every case sorted to a medium size before weighing out for treatment.

Barley (Table 12) was planted on 15 Sept. 1938, sixteen seeds being sown in each box of sand, while three drills were planted with five seeds each in the soil boxes so that these had only fifteen seeds. The sand boxes were watered with standard nutrient solution when the seedlings were about 2 in. high, and subsequently when necessary. Germination counts were made, but there were no significant differences in either final count or rate index.[2] Heights were measured on 27 Sept. and on 10 Oct., but again the treatments were not significant. The seedlings were harvested on 12 Oct., and the roots were washed. The roots and stems were then separated just above the hypocotyl, and were weighed separately for each box. Dry weights were also obtained. Dry weights for stems were analysed and are included in Table 12, but were not statistically significant. Dry weights of roots and fresh weights of both shoots and roots showed obviously that there were no differences, and were not analysed (see Table 12).

[1] Granosan is a proprietary mercurial seed dressing manufactured by the Bayer Semesan Co. in America.
[2] See Appendix.

TABLE 12. *Exp.* 11: *growth data for cereal seedlings—barley*

	Control	Granosan	Granosan + Naphthyl-acetic acid	Granosan + Indolyl-acetic acid	General mean	S.E.	Sig. diff.
Soil: Final count of germination**	100·0 (13·6)	107·4	105·9	105·9	104·8	—	—
Rate index for germination**	100·0 (0·729)	106·1	107·9	108·1	105·5	5·81	17·9
Heights 27 Sept. (cm.)**	100·0 (9·4)	96·6	98·3	101·9	99·2	5·86	18·1
Heights 10 Oct. (cm.)**	100·0 (17·9)	101·4	100·8	104·3	101·6	3·53	10·9
Sand: Final count of germination**	100·0 (16·0)	98·8	95·0	98·8	98·1	—	—
Rate index for germination**	100·0 (0·943)	97·8	97·2	95·9	97·7	1·91	5·9
Heights 27 Sept. (cm.)**	100·0 (10·6)	94·0	96·2	96·6	96·7	3·32	10·2
Heights 10 Oct. (cm.)**	100·0 (19·2)	95·6	96·7	96·4	97·2	2·33	7·2
Soil: Dry wt. of tops, per pot (g.)**	100·0 (1·10)	111·8	112·7	109·1	108·6	7·85	24·4
Sand: Dry wt. of tops, per pot (g.)**	100·0 (1·21)	100·0	98·3	124·8	105·8	17·31	53·9

Oats (Tables 13 and 14) treated in the above manner, were sown on 26 Sept. in the same way as barley. Germination counts were made every day from 4 Oct. Neither final count nor rate index showed any significant differences. The sand boxes were watered with standard nutrient solution at intervals. Heights were measured on 10 and 19 Oct. and subsequently about twice a week. They were analysed statistically up to 22 Oct. and were found not to be significant. Subsequent measurements are given in Tables 13 and 14; but, since it was obvious that the hormonized seed dressings gave figures lower than the control, only the last height measurements were analysed (19 Nov.) and these again were found not to be significant. Tillers were counted on 7 and 11 Nov. and after that were found to increase no more in number before harvest, so that no more counts were completed. The figures are summarized in Tables 13 and 14, but no significant differences were found. The plants were harvested on 21 Nov. at 8 weeks and dry weights were obtained. There were no significant differences. For barley these dressings had no effect.

Wheat (variety Yeoman) (Tables 15–18) was dressed and sown in 1 sq. ft. wooden boxes of soil and sand on 18 Oct. 1938. There were five replicates in sand and five in soil. In the boxes of soil three drills were sown with five seeds in each, fifteen seeds being sown per box. In the sand boxes seeds were sown in separate holes in four rows of four seeds each, sixteen seeds being sown per box. Germination was very slow, owing to the low temperature and, in the case of the soil, to the difficulty of wetting the soil below the surface layer. Soil boxes showed signs of germination 2 days before the sand boxes (1 Nov.). Counts were made every day, but there were obviously no differences between treated and control boxes in either sand or soil. Heights to the tip of the longest leaf were measured from 7 Nov. onwards at intervals gradually increasing to a week, until in December growth in height practically ceased and no more measurements were made until March 1939 (see Table 15). Tillers were counted from the end of November onwards at intervals of a week, until mid-December, and after that at longer intervals (see Table 16). During the whole growth period, the sand boxes were watered at intervals with a nutrient solution containing 300 mg. N/l. During the winter only 250 c.c. were applied at a time to each box at intervals longer than a week, gradually shortening to three times a week in May. From the last week in May the volume given was increased to 400 c.c. per box, and this application three times a week was continued until harvest.

TABLE 13. *Exp.* 11: *growth data for oats: soil*

	Control	Granosan	Granosan + Naphthyl-acetic acid	Granosan + Indolyl-acetic acid	General mean	S.E.	Sig. diff.
Final count of germination**	100·0 (14·0)	101·4	97·1	101·4	100·0	—	—
Rate index for germination**	100·0 (0·832)	108·1	105·5	105·3	104·7	1·76	5·4
Heights (cm.): 10 Oct.**	100·0 (5·88)	104·8	99·3	101·4	101·4	1·62	5·0
19 Oct.**	100·0 (10·0)	99·6	99·4	100·4	99·8	—	—
22 Oct.**	100·0 (11·2)	101·4	101·6	102·7	101·4	—	—
25 Oct.	100·0 (12·91)	98·2	98·1	100·8	—	—	—
28 Oct.	100·0 (14·10)	97·9	97·5	99·7	—	—	—
31 Oct.	100·0 (15·40)	96·1	96·3	99·6	—	—	—
4 Nov.	100·0 (16·59)	95·7	94·75	98·54	—	—	—
7 Nov.	100·0 (17·21)	94·1	94·4	99·2	—	—	—
11 Nov.	100·0 (19·08)	97·6	96·5	102·5	—	—	—
15 Nov.	100·0 (21·6)	97·7	95·8	99·1	—	—	—
19 Nov.**	100·0 (21·9)	98·4	95·3	98·8	98·1	4·95	15·3
Tiller count: 7 Nov.	100·0 (1·43)	84·0	96·5	99·3	—	—	—
11 Nov.	100·0 (1·5)	80·0	93·3	100·0	—	—	—
Dry wt. of tops per box (g.)**	100·0 (1·3)	93·7	95·2	106·3	98·8	15·69	48·3
Dry wt. of roots per box (g.)**	100·0 (0·31)	116·7	126·3	118·6	115·4	7·26	22·4

The sand-grown plants remained much paler in colour than those grown in soil, and up till May were shorter in height, but thereafter they gained on the soil plants and remained taller. The sand-grown plants had fewer tillers at first, but as soon as rapid growth began in March, they increased far beyond the soil plants in number of tillers. In April both lots of plants underwent a sudden decrease in number of tillers, but when ears formed in June, the sand-grown plants had about twice as many ears per box as the soil-grown plants. The dying down of tillers after the spring growth may have been helped by an attack of mildew, which was combatted by spraying with a fungicide, and finally reduced to only a mild growth on the lower parts of the shoots. The plants were later attacked by aphides (7 July) and were sprayed several times with a dilute solution of nicotine during the next few days, and the aphides were thereby practically exterminated. The plants were cropped before they were fully ripe, the grain still being slightly soft. Soil boxes were harvested on 8 Aug: sand on 9 Aug. The shoots were cut off at soil level, the ears cut off, and ears and shoots were weighed separately. The number of ears per box was also counted. The roots were discarded. The fresh and dry weights per box and the number of ears per box are given in Table 17.

TABLE 14. *Exp.* 11: *growth data for oats: sand*

	Control	Granosan	Granosan + Naphthyl-acetic acid	Granosan + Indolyl-acetic acid	General mean	S.E.	Sig. diff.
Final count of germination**	100·0 (15·4)	96·1	96·1	100·0	98·1	—	—
Rate index for germination**	100·0 (0·968)	97·1	100·5	100·4	99·5	1·39	4·3
Heights. 10 Oct. per plant (cm.)	100·0 (6·36)	89·6	99·4	99·1	97·0	2·34	7·2
Heights (cm.): 19 Oct.**	100·0 (11·1)	93·5	102·4	99·1	98·7	2·67	8·2
22 Oct.**	100·0 (11·4)	93·5	103·5	100·7	99·4	2·83	8·7
25 Oct.	100·0 (13·08)	94·1	101·6	99·1	—	—	—
28 Oct.	100·0 (15·18)	92·8	100·1	96·4	—	—	—
31 Oct.	100·0 (16·90)	94·7	101·4	98·4	—	—	—
4 Nov.	100·0 (18·11)	97·0	105·4	101·4	—	—	—
7 Nov.	100·0 (18·87)	95·1	102·3	99·5	—	—	—
11 Nov.	100·0 (22·35)	96·7	102·7	102·5	—	—	—
15 Nov.	100·0 (24·7)	101·2	105·3	105·3	—	—	—
19 Nov.**	100·0 (25·0)	101·5	105·0	105·5	103·0	1·70	5·2
Tiller count: 7 Nov.	100·0 (2·66)	90·6	89·8	95·9	—	—	—
11 Nov.	100·0 (2·7)	96·3	96·3	100·0	—	—	—
Dry wt. of tops per box (g.)**	100·0 (2·1)	84·1	93·5	93·5	92·8	4·55	14·0
Dry wt. of roots per box (g.)**	100·0 (0·74)	85·4	89·2	87·8	90·6	6·79	20·9

TABLE 15. *Exp.* 11: *av. height of wheat per plant*

	Soil: Control	Soil: Granosan	Soil: Granosan + Naphthyl-acetic acid	Soil: Granosan + Indolyl-acetic acid	Sand: Control	Sand: Granosan	Sand: Granosan + Naphthyl-acetic acid	Sand: Granosan + Indolyl-acetic acid
7 Nov.	5·50	5·19	5·31	5·28	3·99	4·04	4·74	4·31
11 Nov.	7·27	7·15	7·04	6·96	5·39	5·57	6·49	5·74
15 Nov.	8·92	8·81	8·82	8·70	6·74	6·67	7·51	6·91
18 Nov.	9·95	9·78	9·89	9·81	8·08	7·79	9·03	8·28
23 Nov.	10·65	10·47	10·53	10·48	9·13	9·51	10·38	9·90
28 Nov.	10·85	11·06	11·21	10·96	9·59	9·51	10·46	9·90
5 Dec.	11·14	11·19	11·47	11·27	9·82	9·62	10·46	9·90
29 Dec.	11·31	11·38	11·48	11·47	10·36	10·75	10·93	10·35
26 Apr.	28·3	26·4	26·6	26·5	25·3	24·7	25·6	25·4
10 May	35·8	33·5	34·2	33·9	32·3	31·9	33·3	32·3
6 June	64·5	60·2	62·4	61·6	67·4	66·8	69·0	65·2
23 June	76·7	75·9	74·8	74·2	88·4	89·7	88·2	85·7

TABLE 16. *Exp.* 11: *tillers and ears*

	Soil boxes				Sand boxes			
			Granosan +				Granosan +	
	Control	Granosan	Naphthyl-acetic acid	Indolyl-acetic acid	Control	Granosan	Naphthyl-acetic acid	Indolyl-acetic acid
28 Nov., tillers per plant	1·63	1·44	1·55	1·51	1·08	1·14	1·15	1·16
5 Dec., tillers per plant	1·84	1·63	1·72	1·76	1·14	1·25	1·51	1·25
12 Dec., tillers per plant	3·37	2·92	3·20	3·15	2·22	2·26	2·64	2·43
24 Jan., tillers per plant	5·13	4·47	5·06	5·00	2·77	3·09	3·54	3·19
9 Mar., tillers per plant	5·14	4·48	5·32	5·08	3·15	3·37	3·81	3·81
24 Apr., tillers per plant	7·4	8·2	7·8	7·3	10·3	10·9	11·2	11·2
11 May, tillers per plant	5·8	6·1	6·0	5·8	8·0	8·0	7·8	8·0
6 June, tillers per plant	2·1	2·3	2·6	2·3	3·3	3·2	3·3	3·4
9 June, ears showing per five boxes	49	22	39	43	71	65	97	80
12 June, ears showing per five boxes	83	65	68	80	141	135	173	143
23 June, ears showing per five boxes	106	114	123	105	197	217	228	201
8 Aug., ears harvested	119	128	131	122	213	225	237	217

TABLE 17. *Exp.* 11: *fresh and dry weights of shoots and ears.* 8 *Aug.*

	Soil boxes				Sand boxes			
			Granosan +				Granosan +	
	Control	Granosan	Naphthyl-acetic acid	Indolyl-acetic acid	Control	Granosan	Naphthyl-acetic acid	Indolyl-acetic acid
Fresh wt. shoots alone (g.)	273·0	266·0	278·0	270·5	515·0	507·5	544·0	508·0
Fresh wt. ears (g.)	131·5	141·5	140·0	136·5	257·5	251·5	278·5	243·5
Av. fresh wt. of ear (g.)	1·105	1·105	1·069	1·119	1·209	1·118	1·175	1·117
Dry wt. shoots alone (g.)	136·9	131·8	140·3	135·0	229·0	231·7	248·6	222·7
Dry wt. ears (g.)	80·8	82·2	85·5	84·8	166·5	152·7	181·8	149·1

TABLE 18. *Exp.* 11: *summary of all statistical analyses made on wheat (figures as percentage of control mean)*

	Soil					Sand				
			Granosan +					Granosan +		
	Control	Granosan	Naphthyl-acetic acid	Indolyl-acetic acid	Sig. diff.	Control	Granosan	Naphthyl-acetic acid	Indolyl-acetic acid	Sig. diff.
Heights: 5 Dec. (cm.)	100·0	100·7	101·4	101·1	6·4**	100·0	98·0	106·5	101·0	10·4**
Tillers: 24 Jan.	100·0	84·4	94·6	94·6	20·4**	100·0	111·3	130·5 +	111·3	12·7
9 Mar.						100·0	107·6	121·7 +	121·0 +	14·4
24 Apr.	100·0	111·4	105·4	99·5	16·2**	100·0	106·0	108·9	109·9	11·0**
Heights: 24 Apr. (cm.)	100·0	93·2	93·8	93·0	11·1**	100·0	97·9	101·6	101·2	10·2**
Tillers: 11 May	100·0	104·8	103·8	100·3	10·4**	100·0	98·8	97·0	98·8	13·1**
Heights: 10 May (cm.)	100·0	93·4	95·5	94·2	11·9**	100·0	98·9	102·4	100·4	9·0**
23 June (cm.)	100·0	98·8	97·5	96·4	7·0**	100·0	101·4	101·2	96·9	4·5**
Ears: 23 June	100·0	107·5	116·0	99·1	28·8**	100·0	110·2 +	115·7 +	102·0	7·9

Statistical analyses were made only when the results appeared to justify further investigation. A summary of these analyses is given in Table 18: nearly all the data failed to give significant differences between treated and control. (Indicated by an asterisk.) Significant differences were found in the sand plants only, for tiller counts (24 Jan. and 9 Mar.) and for ears (23 June), the hormonized seed dressings giving a significantly high count of tillers (especially α-naphthylacetic acid in Granosan) and, in two of these cases, only over the ordinary control and *not* over the Granosan control. For ears, however, non-hormonized Granosan was as good as hormonized (α-naphthylacetic acid) and significantly higher than Granosan × β-indolylacetic acid. Considering that all the other counts of tillers showed no such differences, it seems only fortuitous that α-naphthylacetic acid in Granosan was significantly high in these isolated cases.

Exp. 12: *Winter wheat* (Tables 19, 20)

A small field experiment with strips sown with winter wheat (variety Squarehead's Master) was carried out to test simultaneously all the "hormonized" seed dressings previously tried. Treatments were as follows:

(1) Control—not dressed.
(2) Control—Agrosan G.
(3) Agrosan G + 0·01 % α-naphthylacetic acid.
(4) Agrosan G + 0·1 % α-naphthylacetic acid.
(5) Agrosan G + 1·0 % α-naphthylacetic acid.
(6) Control—not dressed.
(7) Control—talc.
(8) Talc + 0·01 % α-naphthylacetic acid.
(9) Talc + 0·1 % α-naphthylacetic acid.
(10) Talc + 1·0 % α-naphthylacetic acid.
(11) Control—not dressed.
(12) Control—Granosan.
(13) Granosan + 0·4 % α-naphthylacetic acid.
(14) Granosan + 0·4 % β-indolylacetic acid.
(15) Control—not dressed.

A sloping plot of land previously used for potatoes, was the site of this experiment. An area measuring $50 \times 17\frac{1}{2}$ ft. was marked out down the slope. This was bisected lengthwise by a $1\frac{1}{2}$ ft. wide path. The 8 ft. wide strips each side of the path were divided into strips 1 ft. apart so that on each side of the path were 49 strips each 8 ft. long.

The Agrosan G and talc dusts were used at 0·208 % of seed weight, the Granosan dusts were used at $\frac{1}{2}$ oz./bushel, which is equivalent to 0·05 %. These rates, in terms of parts of hormone per million parts of seed weight, are equivalent to:

Agrosan G or talc + 0·01 % hormone ≡ 0·208 p.p.m.
Agrosan G or talc + 0·1 % hormone ≡ 2·08 p.p.m.
Agrosan G or talc + 1·0 % hormone ≡ 20·8 p.p.m.
Granosan + 0·4 % hormone ≡ 2·0 p.p.m.

Thus, the Granosan dusts with 0·4 % hormone were approximately equivalent to the Agrosan G and talc dusts with 0·1 % hormone, owing to the different rates at which they were applied. The seed was dressed the day before it was sown, and was shaken in 40 g. lots in closed glass jars. The rate of sowing was roughly equivalent to 1 bushel/acre: 5·4 g. of seed were sown in each of the 8 ft. drills.

On 4 Nov. 1938, the seed was sown as follows: Two strips at each end of the whole area were sown with undressed seed and were used as buffer rows. The remaining forty-five strips were divided into three blocks, so that on each side of the path there were three blocks of fifteen strips each, corresponding to six replicates of fifteen treatments. The treatments were randomized within each block. The 5·4 g. of treated wheat was sown as evenly as possible along the drill, the seeds being about $\frac{1}{3}$ in. apart. They were afterwards covered by hoeing between the rows. Pegs were placed 1 ft. from both ends of each row. The 6 ft. length lying between these pegs was the only part used for observation, so that the outside 1 ft. long strips acted as buffers.

Germination counts were made daily. The number of seeds per 6 ft. row was calculated to be 81; final counts only reached about 70 in most cases, however. A rate index was worked out from these germination counts. Measurements were later made of heights, and tillers were counted. These data are summarized in Table 19. There are no differences between treated and control strips for any of the counts. Where there was any such possibility, the figures were analysed statistically (see Table 20). The only differences found to be statistically significant were those between the germination rate indices of treatments 5 and 10 and those of the controls. Treatment 5 (Agrosan G + 1·0 % α-naphthylacetic acid) was up, and treatment 10 (talc + 1·0 % α-naphthylacetic acid) was down on the

TABLE 19. *Exp.* 12: *hormonized Agrosan G, Talc, and Granosan on wheat. Summary of growth data*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Height/plant (cm.): 18 Nov.	5·07	5·63	5·01	5·33	5·20	4·97	5·14	4·90	5·06	5·31	5·03	4·70	4·55	4·65	5·03
25 Nov.	8·64	8·21	8·17	7·94	8·67	8·46	8·17	7·82	8·21	8·63	8·21	8·18	7·63	8·10	7·90
1 Dec.	9·74	9·54	9·54	10·18	9·62	10·00	9·54	9·60	9·74	9·29	9·52	9·56	9·42	9·68	9·36
30 Dec.	10·68	10·33	10·90	10·93	11·63	11·42	11·13	11·35	11·44	11·35	11·35	10·83	11·50	11·21	11·75
22 May	87·56	86·52	89·13	88·02	88·31	85·74	87·40	87·04	87·16	86·50	86·32	87·56	87·18	86·86	85·67
Tillers per yd: 24 Feb.*	802	784	743	804	786	739	822	736	796	678	758	771	902	849	681
23 May*	665	618	651	675	638	603	600	602	678	584	618	615	652	593	567
Ears per row: 13 June (=6 ft.)*	1171	1142	1131	1134	1145	1115	1135	1120	1174	1044	1069	1145	1163	1118	1032

* These figures are totals of six replicates.

TABLE 20. *Exp.* 12: *hormonized Agrosan G, Talc, and Granosan on wheat. Summary of statistical analyses made on the data*

	Controls 1, 6, 11, 15	2	3	4	5	7	8	9	10	12	13	14	Sig. diff. A	Sig. diff. B
Rate index % control mean from germination counts	100·0	105·0	102·7	102·0	106·6 +	99·8	98·8	97·8	93·5 −	99·1	99·4	102·0	6·9	5·4
Tillers, 26–28 Apr.**	100·0	101·6	99·1	110·2	107·0	92·6	103·1	104·2	93·6	105·4	112·8	104·1	14·3	11·3
Heights, 26–27 Apr. (cm.)**	100·0	101·8	101·7	103·7	102·2	101·7	103·3	102·0	100·6	102·6	101·3	101·1	5·8	4·6

Significant difference A for comparison of treated mean.
Significant difference B for comparison of control mean *v.* any treated mean.

controls. Thus, it does not appear possible for these differences to be due to the α-naphthylacetic acid: the result must be due to chance. The other data analysed were found to give no significant differences (see Table 20).

Discussion

Templeman (1939) showed that plant-growth substances applied in relatively high concentrations and amounts had no effect on the dry matter production of the plants used. The present paper describes the results of applications of smaller amounts of the active materials. The number of different plants used has been increased, and the majority are of definite agricultural or horticultural value, for which any method of increasing growth would have practical value. As active substances, α-naphthylacetic acid and β-indolylacetic acid have been used on account of their known properties of markedly stimulating roots from stem tissue. It was thought possible that these materials might affect the growth of the plants to which they were applied in two ways: (i) directly, by influencing the growth of the tissues with which, after absorption, they came in contact; and (ii) indirectly, by stimulating the production of roots and so increasing the plant's ability to absorb water and nutrients.

In view of several reports of inconclusive experiments which suggest increase in growth due to growth-substance treatment, it must be pointed out that in our experiments and with the conditions, plants, methods and substances used therein, no striking or consistent increases in growth have been obtained. It might be thought that application by means of seed dressing was unsatisfactory and that little absorption by the plant roots would take place, or that absorption might be only in the very early stages; the "watering-on at interval" technique was used to avoid this possibility and to ensure that growth-substance solution was in contact with the roots throughout the life of the plant. The results show that, in general, the higher concentrations and quantities used have depressed growth and that those below the toxic level have been without effect.

It has often been suggested that organic matter in the soil, by virtue of its growth-substance content, has some direct effect upon plant growth in addition to its important beneficial effects upon physical conditions, water-retaining capacity, etc., of the soil. If such were the case, it is possible that additional applications of growth substance would be ineffective and, accordingly, many of our experiments have been carried out both in soil and in quartz-sand which has been fed with an entirely inorganic nutrient solution. The experiments described in this paper give no evidence that plant-growth substances applied to plants growing in sand or soil, increase plant growth. Other experiments at Jealott's Hill (as yet unpublished) show that excellent crops of economically important plants can be grown in quartz sand in the absence of organic matter, provided due regard is paid to mineral nutrition and water requirements.

The general conclusion to be drawn from these experiments seems to be that so far as our present knowledge of growth substances exists, the plants used are not limited in their growth by their growth-substance contents, but for given levels of light, water, temperature and nutrient conditions, they are able to produce internally their maximum hormone requirements.

Summary

1. All concentrations of sodium-α-naphthylacetate solutions ranging from 0·005 to 33·3 mg. l. applied in pot culture experiments to lettuce, white mustard and tomato, growing in soil and in quartz sand, have not increased growth as measured by plant heights or dry matter production except in one or two isolated instances.

2. Seed dressings containing α-naphthylacetic acid or β-indolylacetic acid in concentrations of from 0·01 to 1·0% in talc or a proprietary fungicide, have not stimulated the germination, tillering, growth in height or dry matter production of oats, barley, sugar beet or wheat grown in sand and soil in pot cultures, and of wheat and oats in small scale field experiments.

Thanks are expressed to Mr R. Jones for help with the routine work of these experiments, to Mr S. J. Lamden for assistance with the statistical computations and to Imperial Chemical Industries, Ltd., for permission to publish this paper.

APPENDIX I

The standard nutrient solution

	mg./l.
$NH_4.H_2.PO_4$	80·9
KNO_3	429·2
$Ca(NO_3)_2.4H_2O$	63·1
$NH_4.NO_3$	65·7
$MgSO_4.7H_2O$	245·5
Hoagland's A–Z minor elements solution	1 c.c./l.

The ratio N : P_2O_5 : K_2O = 1 : 0·5 : 2

APPENDIX II

Rate index

The calculation of a rate index was proposed by Bartlett (1937) for use in interpreting germination counts, the method being to sum the figures for successive counts and divide the total by the product of the final count and the number of counts made.

"This index has no absolute meaning, but since each count includes the count previously made, the number coming up at any date is weighted in accordance with this date's earliness or lateness, so that an efficient analysis of the effect of treatments on rate of germination might reasonably be expected from this index."

In the present paper the same method has been adopted for calculating the rate index of such developments as the formation of trusses on tomato and ears on barley; exactly the same principle being applied, that the counts of trusses or ears for successive dates are summed and divided by the number of counts made multiplied by the figures for the final count.

REFERENCES

Anon. (1939). Hormone or auxin experiment. *J. Dep. Agric. Eire*, **36**, 121.

Amlong, H. U. & Naundorf, G. (1939). Wuchsstoffe und Pflanzenertrag. *Forschungsdienst*, **7**, 465–82.

Bartlett, M. S. (1937). Some examples of statistical methods of research in agriculture and applied biology. *Supp. J. Roy. Stat. Soc.* **4**, 137–70.

Croxall, H. E. & Ogilvie, L. (1940). The incorporation of growth hormones in seed dressings. *J. Pomol.* **17**, 362–84.

HERBST, W. (1939). Wuchsstoffe in der gärtnerischen Praxis. I. Heteroauxin in der Tomatenkultur. *Gartenbauwiss.* **12**, 520–9.

HWANG, Y. & PEARSE, H. L. (1940). The response of seeds and seedlings to treatment with indolylacetic acid. *Ann. Bot., Lond.*, N.S. **4**, 31–7.

KAISER, S. & ALBAUM, H. G. (1939). Early root and shoot growth in two varieties of *Avena sativa* in relation to growth substances. *Amer. J. Bot.* **26**, 746–54.

MCROSTIE, C. P., HOPKINS, J. W. & GRACE, N. H. (1938). Effect of phytohormone dusts on growth and yield of winter wheat varieties. *Canad. J. Res.* Sec. C, **16**, 510–15.

MITCHELL, J. W. (1939). Effect of indolylacetic acid on the growth of some crop plants. *Proc. Amer. Soc. hort. Sci.* **36**, 171–6.

SAYRE, C. B. (1939). Use of nutrient solutions and hormones in the water for transplanting tomatoes and their effect on earliness and total yields. *Proc. Amer. Soc. hort. Sci.* **36**, 732–6.

STIER, H. L. & DU BUY, H. G. (1939). The influence of certain phytohormone treatments on the time of flowering and fruit production of tomato plants under field conditions. *Proc. Amer. Soc. hort. Sci.* **36**, 723–31.

TEMPLEMAN, W. G. (1939). The effect of some plant growth-substances on dry-matter production in plants. *Emp. J. exp. Agric.* **7**, 76–88.

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ADDENDUM

4 *October* 1940

Since this script was sent to Press, the following papers have appeared which give independent and complete confirmation of the results described herein.

BARTON, L. V. (1940). Some effects of treatment of non-dormant seeds with certain growth substances. *Contrib. Boyce Thompson Inst.* **11**, 181–206.

—— (1940). Some effects of treatment of seeds with growth substances on dormancy. *Contrib. Boyce Thompson Inst.* **11**, 229–40.

YOUDEN, W. J. (1940). Seed treatments with talc and root-inducing substances. *Contrib. Boyce Thompson Inst.* **11**, 207–18.

THE CONTROL OF NARCISSUS LEAF DISEASES

II. THE EFFECT OF WHITE MOULD ON FLOWER AND BULB CROP

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(With Plates 19 and 20 and 7 Text-figures)

PRELIMINARY spraying trials and crop records on a commercial scale (Gregory, 1940) indicated the magnitude of indirect losses of narcissus flower and bulb crops from leaf diseases, and the increases which can be obtained when white mould (*Ramularia vallisumbrosae* Cav.) and fire (*Sclerotinia polyblastis* Gregory) are controlled by copper sprays. This paper describes an experiment on narcissus Golden Spur, carried out at the Isles of Scilly Experiment Station to test the effect of attacks of white mould on bulb increase and flower crop over a period of years in a climate favourable to the spread of the disease.

Primary white mould lesions appear on the tips of young leaves soon after they come above ground, and develop white layers of conidia which serve for secondary spread of the organism in moist weather. The infected leaves slowly wither back from the tip, and when dead they are covered with minute black sclerotia which remain dormant on the ground during summer and autumn, but which in winter produce a crop of conidia which causes the primary infections of the new narcissus leaves, thus completing the life cycle (Gregory, 1939). On some farms the old leaves are raked off the surface of the beds and in this way most of the aestivating sclerotia are removed. As normally the fungus does not attack the leaf much below soil level the bulb is clean when lifted, and the incidence of white mould on daffodils in their first season after planting is usually slight.

Experimental treatments were designed to measure the effect on cropping of primary infections at an intensity comparable with that on beds from which infected leaves had not been removed in summer cleaning, and also the effect of secondary infection. As the response of different grades of bulb might differ it was decided to test double-nosed and single-nosed bulbs separately.

ARRANGEMENT AND MATERIAL

An experiment on control by sprays of a rapidly spreading leaf disease is difficult to design. Plots must be large enough to permit satisfactory application of sprays and to allow the disease to develop on untreated plots, but not too large or they become exacting and it is difficult to keep detailed records. On windy days spray and spores drift from one plot to another, and both factors tend to reduce the crop differences between diseased and protected plots. The lay-out finally adopted was a 4×4 Latin square with wide unplanted guard strips between plots.

Field and soil. To get soil as nearly uniform as possible and to avoid land that had been irregularly cropped and manured an old meadow which had remained unbroken for upwards of 14 years was ploughed in the winter of 1936. The field was low-lying and fairly level, with a dark-coloured silty

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granite soil of light texture with pan formation in places. Soil samples from each plot were kindly analysed by Mr A. Blenkinsop at Seale-Hayne Agricultural College, and no obvious gradients were reported. The reaction varied from pH 4·9 to 5·7: potash and phosphates were not considered deficient, the calcium figure was very variable and the magnesium figure low. No manure or fertilizer was applied either as a basal dressing or top dressing before or at any time during the experiment.

Stock of bulbs. The bulbs came from a carefully selected stock of Golden Spur which was free from the rogues so common in this variety. The bulbs had been growing for 3 years in the field next to that selected for the experiment and had suffered the usual attacks of white mould. They were lifted in August 1936 and graded on the ground. Special precautions were taken to mix bulbs from different parts of the field in order to obtain a fair sample. Hot-water treatment of the bulbs against eelworm was not carried out as there was no sign of infestation on this or any adjacent field. The bulbs were a small sample of their grade and averaged: double-nosed, 56 lb. per 1000; and single-nosed, 43 lb. per 1000. Sixteen lots of 240 double-nosed bulbs and sixteen lots of 240 single-nosed bulbs were counted and weighed into trays. The trays were then allocated by random numbers among the different plots.

Planting. Planting was carried out by plough during the last week in October 1936. The entire set of sixteen plots was planted by one man in order to obtain uniformity in depth and position of the bulbs. The furrows were 9 in. apart, each running the length of each column of four plots (Text-fig. 1). Each plot consisted of two beds 20 ft. long, separated by an 18 in. path, containing the double-nosed bulbs on the east side, referred to below as the D/N bed, and the single-nosed bulbs on the west side, the S/N bed. A bed consisted of six rows of forty bulbs set 6 in. from centre to centre. The spacing was that usually adopted in west Cornwall, and wider than favoured in Scilly. Around each plot a 4 ft. 6 in. guard area was left unplanted to reduce drift of spores and spray from one plot to another. On the north and south ends of the block of plots, separated from it by the usual guard strips, was planted the remainder of the same stock of bulbs as that used in the experiment. To the east, beside plots 1, 5, 9 and 13 (Text-fig. 1), was a stone hedge about 4 ft. high, and on the west side lay unbroken meadow. The plots were exposed to the prevailing winds from the south-west. Except for the experimental treatments given, the wider spacing and the presence of unplanted guard strips, all handling and management of the bulbs was carried out according to the usual practices on flower farms in the Isles of Scilly.

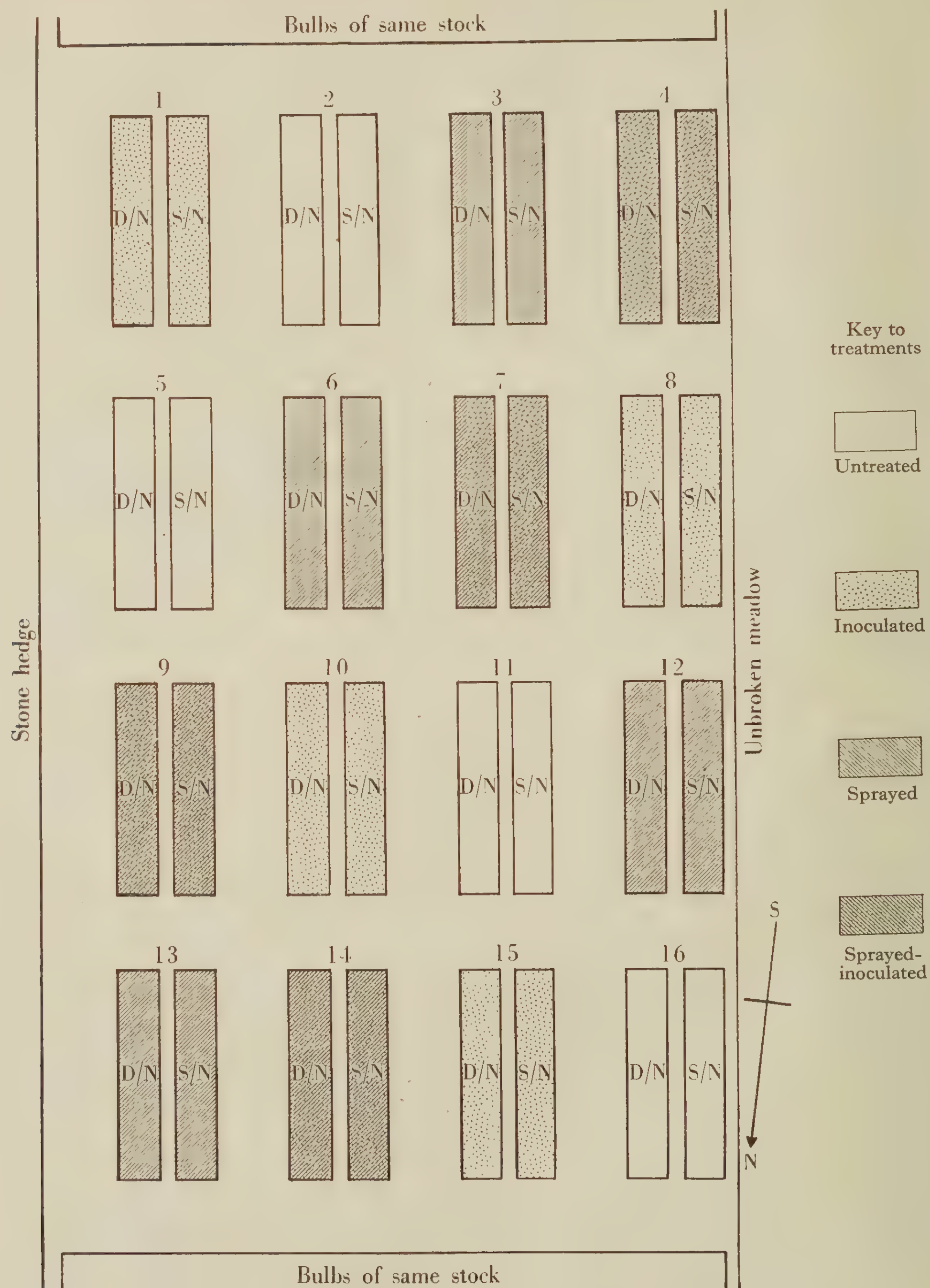
Treatments

The following four treatments, each replicated four times, were planned to test the conditions outlined in the introductory paragraph.

Untreated (plots 2, 5, 11 and 16). No special treatments were given, but the plots were allowed to become attacked by white mould by secondary infection from outside.

Inoculated (plots 1, 8, 10 and 15). To reproduce as closely as possible on newly planted beds the intensity of primary infection from sclerotia on uncleaned beds, these plots were inoculated a few weeks after planting by placing on the ground withered leaves bearing sclerotia of the white mould fungus. The leaves had been collected during summer 1936 by raking beds of the same stock of Golden Spur before lifting. On 10 Dec. 1936, the dried leaves were placed in handfuls somewhat irregularly on the surface of the plots. To reduce the danger of small portions of leaf blowing to other plots the leaf masses were not broken up. In subsequent seasons no further inoculation was carried out, and during the years 1937 and 1938 when leaves on these plots had died down they were raked off in the usual cleaning process.

Sprayed (plots 3, 6, 12 and 13). To control secondary spread of white mould these plots were sprayed when the shoots were about 6 in. high, and again twice after flowering. The formula adhered to throughout was 4–4–40 Bordeaux mixture made with a fresh sample of hydrated lime and about 4–6 oz. of Agral 2/40 gal. added as a wetter. The spray was applied with a pneumatic knapsack machine at 75–50 lb. pressure, at the rate of approximately 200 gal./acre. Spraying was performed three times in 1937 and 1938, but only twice in 1939.



Text-fig. 1. Plan showing arrangement of plots of narcissus Golden Spur in Latin square, with key to treatments, St Mary's, Isles of Scilly, 1936–9.

Sprayed-inoculated (plots 4, 7, 9 and 14). To test the efficiency of Bordeaux mixture in controlling primary infections from sclerotia on the ground this series of plots was given a combination of the treatments received by sprayed and inoculated plots. They were infected artificially in December 1936, and sprayed with Bordeaux mixture in 1937–9. In 1938 and 1939 the spray served to protect the foliage from secondary spread and not, as in the first season, from a heavy primary infection from aestivating sclerotia.

Treatments, 1937. The method of inoculation applied to the inoculated and sprayed-inoculated plots has been described above. Bordeaux mixture was applied to the sprayed and sprayed-inoculated on 4 Feb. 1937, but having been washed off by rain before it was dry, the application was repeated on 5 Feb., and the second effective application was given on 1 Mar. During a prolonged spell of wet weather attempts were made to spray on 13 and 24 Mar. but the spray was removed by rain and a third successful application was not made until 26 Mar.

Observations on foliage, 1937. January and February 1937 were months of exceptionally heavy rainfall in the Isles of Scilly and conditions were very favourable for the spread of white mould. Primary lesions were first noted on inoculated plots on 14 Feb. and by 1 Mar. the disease was spreading on some of these plots, while a few leaf-tip lesions appeared on the sprayed-inoculated plots during the weeks that elapsed between the first and second sprayings. By the third week in March the yellowish brown colour of the inoculated plots seen from a distance indicated a severe attack of white mould. Comparison of the untreated and inoculated plots showed that the attempt to infect beds with aestivating sclerotia had been an outstanding success. At the end of April all the sprayed plots showed striking control of white mould. Inoculated plots were almost completely dead and the untreated plots were intermediate in condition. The differences in health of foliage was clearly shown in photographs taken on 27 Apr. (Pl. 19, figs. 1–4). By 3 June the untreated and inoculated plots were completely withered, but both series of sprayed plots still retained about one-fifth of the foliage alive. These leaves were ripening normally without having become infected with white mould. When the foliage had died down the plots were cut over and the weeds and remains of the narcissus foliage were raked off and burnt. The field was then kept clean by surface cultivations.

Treatments, 1938 *and* 1939. Bordeaux mixture was applied to the appropriate plots on 27 Jan. before flowering, and again after the flower crop had been removed, on 23 Feb. and 28 Mar. 1938, all under good weather conditions. During 1938 more scorching of leaf tips from copper sprays was observed than usual. In 1939 Bordeaux mixture was applied to the appropriate plots on 24 Jan., but rain followed immediately and the application was repeated successfully on 25 Jan. before picking had begun. A second effective application was made on 15 Mar., after the crop had been picked.

Observations on foliage, 1938 *and* 1939. The behaviour of plots undergoing the same treatment was not entirely uniform, but as a result of periodical observations a general summary can be given. Foliage on the sprayed and sprayed-inoculated plots matured slowly during May, the colour first changing from blue-green to yellow-green, and then through yellow to brown as the leaves withered from the tip. In 1937 there had been a marked difference in disease incidence between the untreated and the inoculated plots, but in subsequent years when uniform cleaning operations had been carried out over all the plots, the incidence of disease was similar on both series.

In 1938 and 1939 primary infections were evident on both untreated and inoculated plots by about the middle of February. Secondary spread was rapid in 1938, but in 1939 the disease made little progress until the middle of March. In both seasons the foliage on these plots was completely withered, about 2 weeks earlier than on sprayed plots.

In general, it was estimated that every year the untreated plants lost their foliage approximately 2 weeks earlier than those protected by spraying, and in 1937 the inoculated plots withered some 5 or 6 weeks earlier than the sprayed plots. The effect of these differences in the life of the foliage became apparent during subsequent cropping.

Flower-crop records

Table 1 shows the number of flowers produced by each bed for the years 1937–40, grouped according to treatments, while in Table 2 the crop is summarized under treatments and expressed in the customary flowering percentage, i.e. number of flowers produced each year per hundred bulbs planted in 1936.

Table 1. *Total flower crop per plot each season arranged under treatments*

Treatment	Plot no.	D/N beds 1937	D/N beds 1938	D/N beds 1939	D/N beds 1940	S/N beds 1937	S/N beds 1938	S/N beds 1939	S/N beds 1940
Untreated	2	—	305	394	541	—	238	328	
	5	—	256	292	532	—	244	314	
	11	—	254	340	628	—	191	246	
	16	—	275	413	703	—	232	385	
		425	1090	1439	2404	*465*	905	1273	*2433*
Inoculated	1	—	75	174	437	—	176	196	
	8	—	86	318	585	—	161	259	
	10	—	204	292	519	—	188	217	
	15	—	124	287	529	—	167	260	
		425	489	1071	2070	*465*	692	932	*2212*
Sprayed	3	97	418	569	990	110	238	469	
	6	104	436	670	1078	141	283	550	
	12	97	366	580	1033	106	241	457	
	13	95	390	635	953	115	293	613	
		393	1610	2454	4054	472	1055	2089	*4326*
Sprayed-inoculated	4	123	343	548	881	121	250	451	
	7	154	388	658	1101	103	271	527	
	9	72	374	671	992	119	301	577	
	14	109	430	704	1159	119	267	515	
		458	1535	2581	4133	462	1089	2070	*4160*

Italicized figures estimated from samples only.

Table 2. *Flower number per* 100 *bulbs planted* 1936. *Percentage flowering*

Treatment	D/N beds 1937	D/N beds 1938	D/N beds 1939	D/N beds 1940	S/N beds 1937	S/N beds 1938	S/N beds 1939	S/N beds 1940
Untreated	*44*	114	147	250	*49*	94	120	253
Inoculated	*44*	51	109	216	*49*	72	95	230
Sprayed	41	167	251	422	49	110	217	451
Sprayed-inoculated	48	160	266	430	48	113	209	434

Italicized figures estimated from samples only.

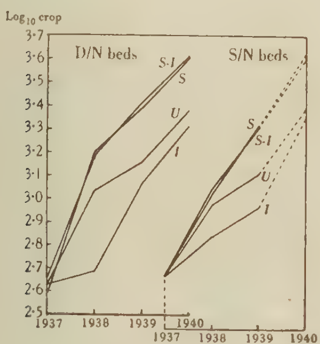
Flower crop, 1937. The flowers produced in 1937 were already present as undeveloped buds when the bulbs were planted in the various plots in October 1936. The crop for 1937 could not therefore have been influenced by the treatments then being started. Complete flower counts were not obtained this season, but where exact counts are not available average figures based on the recorded number of flowers from sprayed plots are given in Tables 1 and 2, and it will be seen that the double-nosed bulbs were a poorer sample of their grade than the single-nosed bulbs.

Flower crop, 1938. The crop in 1938 was affected by the various treatments applied to the plots in 1937. The crop shown in Table 1 is the number of flowers actually picked from each bed. Statistical analysis shows that inoculation had very significantly lowered the yield on sprayed plots, but that among the sprayed plots the slightly lower yields from the sprayed-inoculated series were not signi-

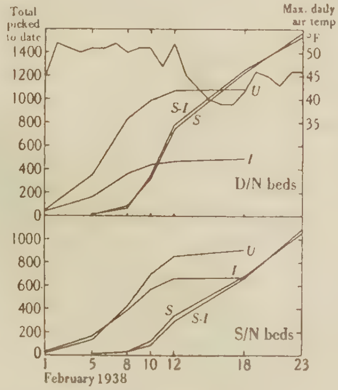
ficant. The increased flower crop from sprayed and sprayed-inoculated series over the untreated plots was highly significant. Both increase from spraying and decrease from inoculation were greater on the D/N beds than on the S/N beds.

Flower crop, 1939. The number of flowers actually picked from the plots is given in Table 1. The differences in crop between untreated plots and sprayed and sprayed-inoculated plots are highly significant, but on account of the rapid recovery in 1938 of the plots which had been inoculated in 1936–7, the effect of inoculation had fallen below the level of significance. The increase due to spraying in this season was more marked on the S/N than on the D/N beds.

Flower crop, 1940. The bulbs were lifted in the summer of 1939, but owing to war conditions it was not possible to replant the produce of all plots separately and to record the crop picked as in previous years. The produce of the S/N plots was planted in two blocks: (1) untreated and inoculated plots; and (2) sprayed and sprayed-inoculated plots. When these bulbs flowered in 1940 the crop from each block was estimated from sample rows, and in Table 1 and Text-fig. 2 the crop has been divided among the treatments in proportion to the weight of bulbs planted. The D/N produce of the D/N beds, on the other hand, were not planted, but during the first week in November 1939 the flower bud was found to be well developed near the neck. Each bulb was cut across with a knife, at several levels if necessary, and the total number of flower buds in the produce of each D/N bed was recorded. At the date when the observations were made this was judged to be an accurate method of assessing crop, as the yellow or green colour of the leaves and the orange yellow of the anthers made the bud stand out conspicuously against the pearly white cut bulb scales. The data are included in Tables 1 and 2.



Text-fig. 2.



Text-fig. 3.

Text-fig. 2. Increase in flower crop from beds planted in 1936 with double-nosed (D/N) and single-nosed (S/N) bulbs, under different treatments during the period 1936–40. U=untreated; I=inoculated; S=sprayed; $S\text{-}I$=sprayed-inoculated. Log$_{10}$ total crop plotted against year. (Figures for S/N beds 1940 estimated from samples only.)

Text-fig. 3. Anthesis 1938. Total flower crop picked up to date from D/N and S/N beds, plotted against date of picking, and showing the retardation of anthesis and larger total crop on sprayed plots. U=untreated; I=inoculated; S=sprayed; $S\text{-}I$=sprayed-inoculated. (With maximum daily air temperature at St Mary's over the period.)

In Text-fig. 2 is plotted log$_{10}$ of mean flower crop from each treatment against season to express the change of crop in terms of growth of the plant. Flower crop gives only an indirect indication of the growth of the plant as a whole because in narcissus part of the increase is expressed vegetatively in the development of new growing points, and only part in the production of new flower buds. The curves indicate several general trends. During the first and second intervals the rate of increase of flower crop is greater on sprayed and sprayed-inoculated than on untreated plots, but during the third interval the rate of increase was not greater as a result of spraying. In 1939 only two spray applications had been given as against

three applications in 1937 and 1938, and it is possible that a third application in 1939 might have led to a bigger rate of increase. As good disease control was obtained by the two applications in 1939, however, it is more probable that the rate of increase fell off on sprayed plots because the larger bulbs, resulting from the cumulative effect of previous sprayings, had reached a stage where competition for space became important. Possibly the slow increase of untreated bulbs during the first two seasons did not bring them to this competitive stage during the course of the experiment.

On the untreated plots the slight incidence of disease in the first season, and its earlier and heavier development in the second season, is reflected in a decline in the rate of increase during the second interval both on the D/N and S/N beds. In 1939 white mould developed more slowly than in previous years on the unprotected plots, and this may account for the rate of increase during the third interval being greater than during the previous year. After the initial effect of early heavy primary infection the inoculated plots showed a marked power of recovery, and the rate of increase exceeded even that of sprayed plots in the same seasons, though this rate was on a smaller capital. Inoculation had its greatest effect in depressing rate of increase during the first interval on the D/N beds and during the second interval on the S/N beds.

Anthesis

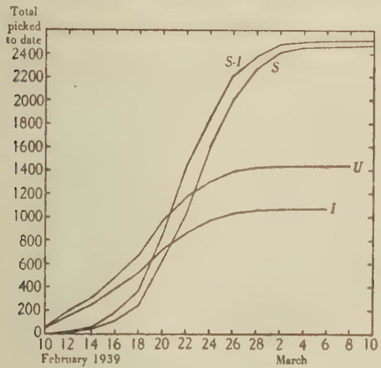
Preliminary work had shown that spraying Golden Spur, besides increasing the crop, delays flowering the following year. This retardation phenomenon was studied in detail in this experiment.

1938. When the plots were examined in January 1938 considerable variation was noted in the forwardness of the leaves. Part of this variation was obviously due to local conditions: in particular, plants near the hedge on the east side of the field were forward. But this positional variation was much less noticeable than differences between treatments. Judging by the height of the foliage above soil level two effects were observed: (1) sprayed plots were retarded in comparison with unsprayed plots; and (2) the beds planted with single-nosed bulbs in each plot were less forward than the beds planted with double-nosed bulbs. These differences are shown in photographs taken on 27 Jan. (Pl. 20, figs. 1–4).

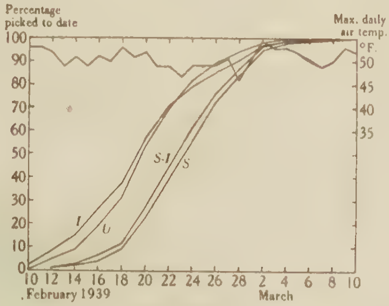
Records of the numbers of flowers picked from each bed on different dates during the season show that the retardation of the plant observed in leaf development was maintained throughout the period of flowering. On untreated and inoculated plots picking began on 1 Feb., reached its maximum on 8 Feb. and lasted until 18 Feb. On the sprayed and sprayed-inoculated plots, except for a few early flowers, picking did not begin until 5 Feb. and lasted until 23 Feb. with a maximum pick on 18 Feb. This retardation is shown graphically in Text-fig. 3, where the total crop picked up to certain dates is plotted for each of the four treatments. As a measure of retardation the date by which 50% of the crop matures may be taken as a convenient index, and will be referred to as the "half-crop date". In practice this date is approximately that of the biggest pick. It will be seen that on the D/N beds spraying had delayed the half-crop date by about 6 days, while on the corresponding S/N beds the delay was as much as 8 days. There is good evidence, however, that in this season the retardation had been exaggerated by a spell of cold weather at the peak of the cropping of the sprayed plots, lasting from 13 to 17 Feb., during which the daily maximum

air temperature fell over 10° F. This is shown by the temperature records on Text-fig. 3 for St Mary's of the period of cropping.

1939. In 1939, as in 1938, differences in earliness were apparent as the leaves came above ground. To obtain a more accurate measure of the differences in anthesis, the rate at which each bed matured its crop was determined by standardizing: (1) the time interval between successive pickings; and (2) the stage of development at which each flower was picked. Picking was carried out at intervals of approximately 48 hr., between 2.0 and 5.0 p.m. every second day. At each picking there were removed from the plots all buds which had swollen sufficiently to separate the claw which holds together the three outer perianth lobes. This stage is slightly later than that selected as most suitable for commercial picking in the Isles of Scilly, but was chosen to increase precision of the records because it is a definite and easily recognizable stage. The count obtained at each picking represents, therefore, the number of



Text-fig. 4.



Text-fig. 5.

Text-fig. 4. Anthesis 1939, D/N beds. Total flower crop picked up to date plotted against date of picking with stage of development and time interval standardized, showing retardation of anthesis and larger total crop on sprayed plots. U=untreated; I=inoculated; S=sprayed; S-I=sprayed-inoculated.

Text-fig. 5. Anthesis 1939, S/N beds. *Percentage* of total crop picked to date at standardized time interval and stage of development, plotted to show retardation on sprayed plots. (With maximum daily air temperature at St Mary's over the period.) U=untreated; I=inoculated; S=sprayed; S-I=sprayed-inoculated.

buds which had reached the stage of claw separation during the preceding 48 hr. period. After picking, the bunches from each bed were weighed on a balance sensitive to 1 g., and the number of flowers counted. Picking began on 10 Feb. and continued until 10 Mar. It was evident that a few flowers on very early beds, plot 1 D/N and plot 5 D/N, had been ready for picking a day or two before records started, and this accounts for the high total recorded from inoculated and untreated series on 10 Feb. The totalized flower crop from the D/N beds plotted against date of picking is shown in Text-fig. 4. For the S/N beds the totalized *percentage* crop picked to date is plotted against date of picking in Text-fig. 5. The percentage crop is here used in place of the customary total crop because in this experiment large differences in total crop between treatments obscure the true magnitude of the retardation. Text-figs. 4 and 5 show that the half-crop date for the various treatments is approximately as follows:

Untreated and inoculated	D/N beds	18 Feb.
	S/N ,,	20 ,,
Sprayed and sprayed-inoculated	D/N ,,	22 ,,
	S/N ,,	23 ,,

The retardation due to spraying was thus about 3 or 4 days, or only half as great as that recorded in 1938. During the whole period of cropping in 1939 the maximum daily air temperature at St Mary's, as shown in Text-fig. 5, was remarkably uniform, and unlike 1938 no sudden cold spell intervened to exaggerate the retardation. It is probable therefore that the true retardation of anthesis due to spraying the stock of Golden Spur with Bordeaux mixture averaged about 4 days.

1940. In 1939 the bulbs were lifted and stored in trays in a loft until October when the produce of all sprayed and sprayed-inoculated S/N beds was planted in one block, and the produce of the untreated and inoculated S/N beds in an adjacent strip. Observations made on foliage and buds in the first week in February 1940 showed that the differences in anthesis due to spraying had not been masked by lifting and storing the bulbs out of the ground. Few of the flower buds on sprayed bulbs were more than 1–2 in. above ground, while some buds on the unsprayed bulbs had already burst the spathe.

The effect of this retardation in lowering the value of the crop was studied in 1938 and 1939. On the basis of actual salesmen's returns for narcissus Golden Spur from the Experiment Station at different dates in the season the value of the flowers from the different treatments was calculated as shown in Table 3. In 1938 the effect of retardation in lowering the average value per bunch from sprayed plots has been more than compensated for on the D/N beds by the higher gross return from the larger flower crop. On the S N beds, however, where crop increase from spraying was not so great, the gross return was actually lower than from the unsprayed beds. The value for 1939 calculated in the same way is also shown in Table 3. In this season, in spite of the retardation of sprayed plots having lowered the average price per bunch, the increased flower crop has led to higher returns on both D/N and S/N beds.

TABLE 3. *Estimated value of flowers from treatments. Estimated effect of treatment on value of flowers*

	1938				1939			
	Total value shillings		Av. pence per bunch		Total value shillings		Av. pence per bunch	
	D/N	S/N	D/N	S/N	D/N	S/N	D/N	S/N
Untreated	47·5	35·7	6·05	5·94	37·8	31·4	3·78	3·57
Inoculated	20·7	27·5	6·10	5·74	28·6	24·4	3·83	3·79
Sprayed	50·6	32·2	4·64	4·40	53·2	45·6	3·11	3·12
Sprayed-inoculated	50·5	34·5	4·75	4·45	55·8	46·1	3·20	3·17

It is possible that when extra costs of processing and marketing the larger number of flowers from sprayed plots are taken into account the increase from spraying would not be worth while. Against this, however, must be set the improved quality of flowers from sprayed plots. Evidently the retardation effect is a serious factor to be considered in the control of leaf diseases of narcissus by spraying.

Flower quality

Flower quality is elusive: it can be assessed by an experienced buyer but is difficult to measure. In order to obtain some indication of the quality of flowers produced under the various treatments the weight to the nearest gram, as well as the number of flowers from each bed at each picking was recorded from 10 to 28 Feb. 1939 inclusive, comprising all but the tail-end of the crop for that season. The records show that the average weight per flower was 15% heavier from sprayed than from unsprayed plots. Text-fig. 6 shows how the mean flower weight varied from day to day from the different treatments. It is clear that on both the D/N and S/N beds of sprayed plots flowers were consistently heavier than on the unsprayed plots, and that the flowers from the D/N beds were generally heavier

than those from the corresponding S/N beds. Analysis of variance carried out on the mean weights per plot during the season showed the increase in flower weight to be highly significant (Table 4).

TABLE 4. *Average weight (g.) per flower (10–28 Feb. 1939)*

Treatment	Plot no.		D/N beds	S/N beds
Untreated	2		6·99	7·18
	5		7·40	6·74
	11		7·29	6·52
	16		7·21	6·46
		Av.	7·22	6·72
Inoculated	1		7·32	6·29
	8		7·30	6·17
	10		7·18	6·79
	15		7·35	6·48
		Av.	7·29	6·43
Sprayed	3		7·81	6·93
	6		8·99	8·23
	12		7·85	7·27
	13		8·95	8·36
		Av.	8·40	7·70
Sprayed-inoculated	4		7·07	6·27
	7		8·64	7·59
	9		8·93	8·06
	14		8·35	7·70
		Av.	8·25	7·45

In order to carry further the analysis of flower quality, on 20 Feb. samples of fifty flowers were taken m each of the beds in the four plots 2, 6, 10 and 14, forming one complete column of the Latin uare. The flower heads were removed from the stem by cutting through the base of the spathe. ower head and stem were then weighed separately, and the length of each stem was measured. The an figures per plot are shown in Table 5. The data are not sufficiently extensive for statistical atment but are regarded as an indication that the extra weight of flowers from spraying is shared both stem and flower head. There was no indication that the stems from sprayed plots were longer an from the unsprayed, but the extra stem weight was visibly due to a greater thickness (shown in ble 5 as weight per unit length). The improvement in flower quality from spraying revealed by the ta of Table 4 can be summed up as a highly significant increase of about 15% in total weight, which able 5 shows to be made up of heavier flower heads and sturdier stems.

TABLE 5. *Mean weights of flower-head and stem of samples from four plots (20 Feb. 1939)*

		Mean wt. flower (g.)		Mean wt. stem (g.)		Mean length stem (cm.)		Mean wt. stem per 1 cm. (g.)	
Treatment	Plot no.	D/N	S/N	D/N	S/N	D/N	S/N	D/N	S/N
Untreated	2	2·04	2·16	4·90	5·18	27·1	28·0	0·181	0·185
Inoculated	10	2·04	2·02	5·14	4·58	28·1	27·1	0·183	0·169
Sprayed	6	2·40	2·38	6·51	5·95	31·1	29·7	0·209	0·200
Sprayed-inoculated	14	2·38	2·28	6·49	5·09	29·3	26·7	0·221	0·191

BULB LIFTING

Lifting was begun on 5 June 1939 in ideal weather. The bulbs were ploughed out of the rows, and after most of the adhering soil had been shaken off were put into trays, taken under cover and weighed on a spring balance sensitive to 1 lb. The weights lifted from the different beds are shown in Table 6. The yields from the sprayed and sprayed-inoculated series of

plots averaged about 80% heavier than from the untreated series. After the preliminary weighings the trays were stored in piles on the floor of a dry and fairly well-ventilated loft. In order to discover whether this big increase in yield on the sprayed plots was maintained throughout the period of storage, or whether loss of weight by drying and respiration of the bulbs would be greater in the produce of the sprayed plots, the bulbs were again weighed at intervals on several occasions. By 7 Nov. the average loss of weight from bulbs lifted from the D/N beds was as follows: untreated, 20·3%; inoculated, 19·7%; sprayed, 17·2%; sprayed-inoculated, 19·8%. The data for the S/N beds were also similar up to 18 Aug., beyond which no further weighings were made on this series. The data obtained show conclusively that as indicated in a previous paper (Gregory, 1940) the relative bulb weights at lifting or at any other time during storage may be taken in assessing the effects of treatments.

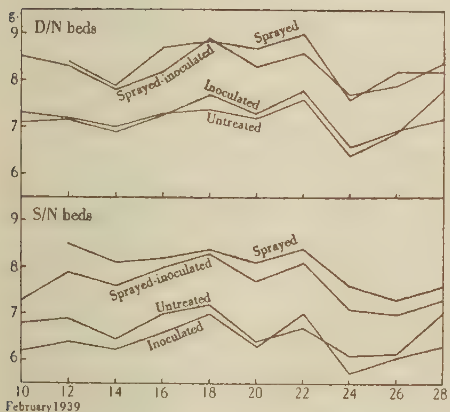
TABLE 6. *Net bulb weights (lb.) 1936 and 1939 by treatments*

		D/N beds			S/N beds		
		1936	1939		1936	1939	
Treatment	Plot no.	Oct.	5–10 June	18 Aug.	Oct.	5–10 June	18 Aug.
Untreated	2	13½	42	36	10½	43	37
	5	13	38	33	10	36	32
	11	14½	45	40	10	32	28
	16	12½	52	45	10½	43	37
		53½	177	154	41	154	134
Inoculated	1	14½	31	28	10	26	23
	8	13½	48	42	10	37	34
	10	13½	39	35	11	35	31
	15	14	39	35	10½	34	30
		55½	157	140	41½	132	118
Sprayed	3	12	80	70	10	58	50
	6	13	81	73	10½	63	56
	12	13½	89	79	10	60	51
	13	13	75	66	10	64	57
		51½	325	288	40½	245	214
Sprayed-inoculated	4	14	81	67	10½	57	48
	7	13	84	74	10½	64	57
	9	12½	73	64	10	62	54
	14	14	79	72	10½	60	53
		53½	317	277	41½	243	212

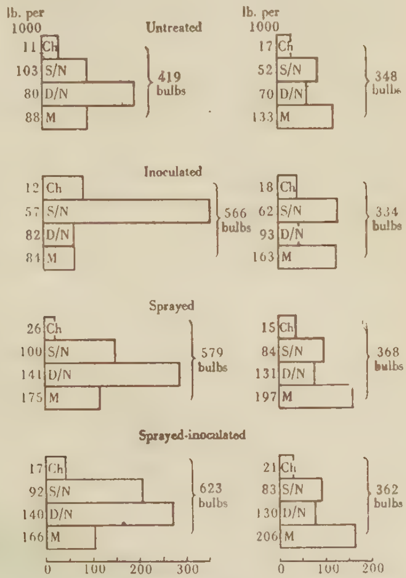
For calculation the weights recorded on 18 Aug. (Table 6), after 10 weeks' storage, were taken for analysis of variance, as representing bulbs in the normal condition for marketing. Statistical analysis shows that the difference in weight between sprayed and sprayed-inoculated plots on the one hand, and untreated plots on the other, is highly significant for both D/N and S/N beds. The effect of inoculation which had depressed flower-crop to such a marked extent in the following seasons was significant only at the 5% level in the D/N series and was non-significant in the S/N beds, again demonstrating the power of rapid recovery.

Grading of bulbs. The foregoing study showed net weight yields which were about 80% heavier on the sprayed than on the untreated plots, and a reduction of about 10% on the inoculated plots. Additional data were obtained by grading the produce of plots 1–8, counting and weighing each grade. Grading of plots 9–16 could not be completed owing to

war conditions, and the incomplete data cannot therefore be subjected to statistical analysis, but are given here as an indication of the various treatments upon the grade and size of bulbs lifted. The bulbs were graded into "chips", "single-nosed", "double-nosed" and "mother-bulbs". The eight plots graded included two from each of the four treatments, and in Text-fig. 7 and Table 7 the data are presented as the means of corresponding plots under-



Text-fig. 6.



Text-fig. 7.

Text-fig. 6. Mean flower weights (g.) 1939 from D/N and S/N beds under different treatments, at each picking over the period 10–28 Feb. inclusive.

Text-fig. 7. Diagram showing number of bulbs of each grade lifted in 1939 from D/N and S/N beds under different treatments, together with total number of bulbs and weights in lb. per 1000. Grades: Ch = chips; S/N = single-nosed; D/N = double-nosed; M = mother bulbs.

going the same treatment. The total number of bulbs lifted was approximately double the number planted. Text-fig. 7 shows diagrammatically the number of bulbs of each grade lifted as a result of various treatments, and also the weight per thousand of this grade. In Table 7 are compared the number of noses and weight per thousand noses in order to show the effect of treatments upon the development of growing points. In general, the most

TABLE 7. *Effect of treatments on number of bulbs and growing points lifted (means of two plots)*

	No. of bulbs		Total no. of noses		Average wt. (lb.) per 1000 noses	
Treatment	D/N beds	S/N beds	D/N beds	S/N beds	D/N beds	S/N beds
1936: Bulbs planted	240	240	480	240	28	43
1939: Untreated	419	348	803	805	42·9	42·8
Inoculated	566	334	757	649	46·9	43·8
Sprayed	579	368	1113	802	64·2	66·0
Sprayed-inoculated	623	362	1110	807	63·6	64·9

striking effect of spraying has been to increase the average weight of double-nosed and mother bulbs lifted, a result that accords well with results obtained in a previous study of this variety of narcissus (Gregory, 1940).

The effect of spraying on the number of "noses" was calculated from the grading data of the various plots, and the data shown in Table 7 include for comparison relevant figures for the bulbs when planted in 1936. The most striking effect is that the bulbs from both D/N and S/N beds of the untreated plots gave the same total number of noses. Inoculation had decreased this slightly in both D/N and S/N beds. Spraying had no effect on the number of noses lifted from the S/N beds, but had increased by 37% the number of noses lifted from D/N beds. The average weight per nose is also of interest, and shows clearly that the main effect of spraying was to enable more reserves to be accumulated by each growing point. The weight per 1000 noses from both D/N and S/N beds is very similar according to treatment. Spraying has increased the average nose weight by 46%.

Interpretation

The differential effect of treatments upon the two grades planted can be explained in terms of the studies carried out by Huisman & Hartsema (1933) on narcissus King Alfred. A growing point may produce leaves and grow in size for several years before it terminates in a flower, which with one subtending leaf, is enclosed by two sheathing leaves. It is then replaced by a new main bud, in the axil of the inner sheathing leaf, which flowers in the next season. A secondary bud is also developed in the axil of the outer sheathing leaf, and this remains dormant for a year before producing leaves for 2 or 3 years and ultimately reaching flowering size. The main bud thus normally gives rise to a flowering bud each year, but its secondary buds take from 3 to 4 years to develop to flowering size. The size of the main bud is regularly limited by flower production, but the secondary bud in its vegetative years accumulates all reserves as capital until it reaches flowering size. The effect of spraying is probably manifest soonest on secondary buds which can accumulate reserves and reach flowering size more quickly, and also on non-flowering main buds which have been dwarfed by disease, close-spacing or infrequent lifting.

A possible interpretation is given in Table 8 which indicates the changes known or assumed to have taken place between 1936 and 1940. There was of course considerable diversity in behaviour on the part of individual bulbs, but the table indicates average trends. The interpretation assumes that there is an average limiting weight of reserve per nose, below which a bud does not produce a flower, and that one bud cannot drain the reserves of another bud.

Table 8. *Interpretation of observed and assumed grade changes* 1936–9

	Untreated		Inoculated		Sprayed	
Season	D/N	S/N	D/N	S/N	D/N	S/N
1936–7	D_0+D_1	S_0+S_1	D_0+D_1	S_0+S_1	D_0+D_1	S_0+S_1
	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓
1937–8	D_1 D_1	S_1 D_1	D_0 D_1	S_0 S_1	D_1 D_2	S_1 D_1
	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓
1938–9	D_1 M_2	D_1 M_2	D_1 D_1	S_1 D_1	M_2 M_3	D_2 D_2
	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓
1939–40	M_2 D_2	M_2 D_2	M_1 S_1	M_2 M_2	D_2 M_3	M_3 D_2
	+ +	+	+ +	+	+ +	+
	S_1 D_2	S_1	S_1 S_1	S_0	D_2 S_1	S_1

In the diagram D represents a double-nosed bulb, S represents a single-nosed bulb, and M a mother bulb. The suffix indicates the number of flowers present in the bulb.

1936–7. In 1937, of the bulbs planted in 1936 as small double-nosed and small single-nosed, only about 50% flowered, so the bulbs of each grade, although of the same apparent stage of development, must have been of two kinds in approximately equal numbers, which may be represented as D_0–D_1 in the D/N beds, and as S_0–S_1 in the S/N beds according as the main bud was or was not large enough to flower. The subsequent development is discussed below and represented diagrammatically in Table 8.

1937–8 (effect of 1937 treatments). *Untreated plots:* both D/N and S/N beds yielded approximately one flower per bulb, so that presumably the main buds had by now all reached flowering size but secondaries had not done so. *Inoculated plots:* on the D/N beds, as in the previous season, only about 50% of the bulbs produced a flower, and it is probable that these were from the main buds; in the S/N beds the depressing effect of inoculation was not so marked as in the D/N possibly because the higher average weight per nose of the single-nosed bulbs when planted enabled more main buds to reach flowering size. *Sprayed and sprayed-inoculated plots:* in the D/N beds on the average each plant produced one flower from the main bud, and in 50% of the bulbs secondaries which had shown leaves for about two seasons previously (graded D/N in 1936) had also reached flowering size; in the S/N beds the plants tended to produce one flower from each main bud, but the secondaries which were younger than those in the D/N beds had not gained enough by spraying during one season to bring them up to flowering size.

1938–9 (effect of 1938 treatments). *Untreated plots:* both the D/N and S/N beds produced approximately one flower from each main bud, and in addition one flower from half of the secondary buds. *Inoculated plots:* both D/N and S/N beds averaged one flower per bulb, probably the 50% non-flowering main buds of the previous season had now acquired a large enough reserve to reach flowering size. *Sprayed and sprayed-inoculated plots:* in the D/N beds all the main buds and all secondaries visible as noses at planting had now reached flowering size, and in addition on 50% of the bulbs a new secondary, not visible as a nose on planting, also flowered for the first time.

1939–40 (effect of 1939 treatments). *Untreated plots:* most D/N bulbs planted produced on lifting after three years either two small double-nosed bulbs, each with two flowers, or a large single-nosed bulb with one flower and a mother bulb with two or three flowers; most S/N bulbs planted, on the other hand, produced either a double-nosed plus a single-nosed bulb, or one mother bulb. *Inoculated plot:* most D/N bulbs planted produced either one mother bulb and one single-nosed bulb giving two flowers between them, or two single-nosed bulbs each with one flower. The effect of inoculation had been to increase the proportion of single-nosed bulbs lifted at the expense of the double-nosed. In the S/N beds most plants produced either one mother bulb, or one single-nosed and one mother bulb. *Sprayed and sprayed-inoculated plots:* most D/N bulbs planted produced either two double-nosed bulbs with two flowers each, or one mother bulb and one single-nosed bulb with three flowers between them; while most S/N bulbs planted produced either one mother bulb or one double-nosed and one single-nosed bulb.

The effects of the treatments in terms of the untreated plots may be generalized as follows. The inoculated plots made no progress during the season following the artificial infection, but during the two subsequent seasons when normal cleaning operations were carried out they almost caught up with the untreated plots. Sprayed plots were able to make about as much growth in two years as the untreated plots made in three. As a practical deduction from these results the importance of cleaning all dead foliage from the beds cannot be over-emphasized.

Discussion

The delayed anthesis on sprayed plots of Golden Spur is apparently about 4 days, and the data given show that in the earliest districts it has a marked effect on the value of the crop. The retardation affects not only the opening of the flower, but is noted earlier as a delay in

development of the leaves. The phenomenon is worthy of further study in view of the large gains in crop which could be obtained by spraying of Golden Spur. In the light of crop records it is now possible to criticize the planning of the experiment. The significance of variances for columns recorded in several instances may indicate that the nearness of the stone hedge on the east side of the plots and of a meadow on the west had provided an infection gradient parallel with the columns. This was unavoidable in the space available, and merely reduced the sensitiveness of the lay-out to an unimportant extent in view of the large treatment differences observed. Further, the differences between treatments were so large that it was not necessary to weigh the bulbs before planting, and it is now clear that time might have been saved by marking out plots containing equal numbers of bulbs on any uniformly graded stock in its first year after planting. The fact that infection crossed the guard strips from inoculated to untreated plots shows that the gap left was inadequate, and instead of the untreated plots remaining practically free from infection in the first season they acquired a light intensity of infection even in their first year. To this extent the conditions of the experiment were artificial and this must have tended to exaggerate the differences recorded in 1938 between untreated and sprayed plots. The adoption of the wider spacing practised in west Cornwall, rather than the close planting usual in the Isles of Scilly, was fortunate in indicating that commercial planting practices in different areas need critical comparison. In 1939 bud counts were made on parts of the same stock of Golden Spur adjacent to the plots but not included in the experiment. Table 9 compares the yield of flowers per 1 ft. run of bed from the close-planted commercial stocks with the average yield from the various experimental treatments.

TABLE 9. *Average flower crop per 1 ft. run of bed* 1939

Treatment	Wide spacing (12 bulbs per 1 ft. run bed) D/N	S/N	Close spacing (21 bulbs per 1 ft run bed mixed flowering grades)
Untreated	18·0	15·9	18·8
Inoculated	13·4	11·7	—
Sprayed	30·6	26·1	—
Sprayed-inoculated	32·2	25·9	—

The relatively high yield per unit area obtained with the wider spacing indicates that where close spacing is adopted with a 3-year rotation competition between plants is probably severe. Observations showed that some of the bulbs never reached flowering size during the 3 years. It is possible that to obtain maximum increases from spraying and other control measures wider spacing will be essential, and evidence given on p. 477 indicates that on the plots the rate of increase on sprayed beds was beginning to fall off by the third year on account of competition between plants. A complex experiment on the interaction of spray programme, spacing and rotation is now needed to determine under what conditions maximum crop can be obtained.

The experiment has demonstrated the effect of white mould upon the flower and bulb crop at four levels of infection intensity in 1937 which were in decreasing order:

Inoculated plots: heavy primary and heavy secondary infection.

Untreated plots: negligible primary and light secondary infection.

Sprayed-inoculated plots: light primary and negligible secondary infection.

Sprayed plots: negligible primary and negligible secondary infection.

In 1938 and 1939 there were only two effective intensities of infection over the plots as a whole:

Unsprayed plots: light primary and heavy secondary infection.

Sprayed plots: negligible primary and negligible secondary infection.

In basing practical recommendations on the results of the experiment it is necessary to bear in mind the limits under which the work was carried out: a 3-year rotation; planting of small bulbs of each grade; the presence of four levels of infection intensity; spacing; and effects of soil and season that can never be exactly repeated.

Summary

1. In 1936 an experiment with narcissus var. Golden Spur was started in the Isles of Scilly in the form of a 4×4 Latin square to measure the effect of flower and bulb crop of controlling primary and secondary infection of the foliage by the white mould fungus, *Ramularia vallisumbrosae* Cav.

2. Judged by the flower crop in 1938, plots which had been exposed to severe primary infection in 1937 from sclerotia on old leaves had made no increase, but in subsequent years showed marked recovery when infected leaves were cleaned off the beds.

3. Plots sprayed with Bordeaux mixture showed large increases of flower crop in comparison with untreated plots, an effect which was cumulative from year to year. Sprayed plants were able to make about as much growth in two years as untreated plants did in three.

4. Flowers from sprayed plots were of better quality and in 1939 were significantly heavier than those from unsprayed plots.

5. Spraying was followed by a marked retardation of leaf growth and anthesis on the same plot in the following season. The retardation varied from 6 or 8 days in 1938 to 4 days in 1939, and its effect was to diminish the value of the increased crop obtained by spraying.

6. After three years the bulb yield from sprayed plots was 80% heavier than that from untreated plots. The increase consisted principally in a greater average bulb weight of the double-nosed and mother bulbs. The number of bulbs lifted was not increased by spraying, neither was the number of noses increased in the produce of beds planted with single-nosed bulbs, but the spraying of beds planted with double-nosed bulbs had increased the number of noses by one-third.

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REFERENCES

GREGORY, P. H. (1939). The life history of *Ramularia vallisumbrosae* Cav. on narcissus. *Trans. Brit. mycol. Soc.* **23**, 24–54.

—— (1940). The control of narcissus leaf diseases. I. White mould and fire on "Golden Spur". *Ann. appl. Biol.* **27**, 338–47.

HUISMAN, E. & HARTSEMA, A. (1933). De periodieke ontwikkeling van *Narcissus pseudonarcissus* L. *Meded. LandbHoogesch. Wageningen*, **37**, 3–53.

EXPLANATION OF PLATES 19 AND 20

PLATE 19

Figs. 1–4. Photographs showing condition of plots 1–4 on 28 Apr. 1937. Plot 1, inoculated; plot 2, untreated; plot 3, sprayed; plot 4, sprayed-inoculated.

PLATE 20

Figs. 1–4. Photographs showing differences in development of foliage on sprayed and unsprayed plots on 27 Jan. 1938 (season following treatments illustrated in Pl. 19, figs. 1–4). Plot 1, inoculated; plot 2, untreated; plot 3, sprayed; plot 4, sprayed-inoculated.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

GREGORY.—The Control of Narcissus Leaf Diseases (pp. 472–488)

Fig. 2

Fig. 4

Fig. 1

Fig. 3

GREGORY.—The Control of Narcissus Leaf Diseases (pp. 472–488)

BOTRYTIS DISEASE OF LETTUCE, ITS RELATION TO DAMPING-OFF AND MILDEW, AND ITS CONTROL BY PENTACHLORO-NITROBENZENE DUST

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(With Plate 21 and 1 Text-figure)

BROWN (1935) recorded preliminary observations on the *Botrytis* disease of lettuce, together with an account of 2 years' experiments in which the efficiency of a fungicidal dusting method was tested. This work has been continued and expanded over the last 4 years and is described in this paper. It has become clear that the *Botrytis* problem, as it affects lettuces in frames, is not a simple problem arising from the attack of one fungus, viz. *Botrytis*, but that others—at least two species of damping-off fungi and *Bremia*—contribute to the general effect, causing a certain amount of direct loss and acting as precursors to the more serious *Botrytis* disease.

EXPERIMENTAL METHODS

The practical methods of lettuce cultivation were outlined in the paper cited above, so that it is merely necessary to state that the work relates to "Spring" lettuce, i.e. to lettuce sown in cold frames in the autumn (October–November) and planted into open ground in early spring (February–March or April). While the routine management of frames followed the usual commercial practice, conditions in the earlier years were purposely arranged to favour development of the disease and thereby to afford a drastic test of the efficiency of the fungicidal method. Thus, crops of seedlings were grown year after year in the same frames and same soil, sowings were dense and the seedlings were unthinned, in each year at least one sowing was made well ahead of the date which growers consider to be safe for this purpose, and the frames were not ventilated as freely as is done by the better growers. Later, when it had been shown that disease could be well controlled under these unfavourable conditions, the standard of cultivation was raised by additions of fresh compost or fertilizers (potassium, phosphorus) to the top-soil of old frames and by the setting up of new frames, by less dense sowing (1·8 g. seed/sq. yd.), by thinning of the seedling crops when necessary and by freer ventilation in open weather. The practical object being to obtain early maturing crops, it was obvious that much of the advantage of early sowing would be lost if the seedlings were undersized or spindly on account of intense crowding.

The fungicide used was pentachloronitrobenzene (referred to as Pcnb) in the form of a dust containing 80% of talc or slaked lime as filler,[1] which was applied in several ways. It was incorporated in the top-soil of frames immediately before sowing (1 oz./sq. yd., worked in to a depth of about 2 in.), or dusted on the surface just after the seed was covered. This was for the purpose of controlling damping-off. In addition, the stand of seedlings was dusted a number of times throughout the winter, the final treatment being given immediately before the plants were pulled for transplanting to the open ground. The dust was applied either by blowing it into closed frames or by traversing the stand of plants in open frames with a blower. The former method lends itself better to quantitative treatment and as such was largely adopted in the later years of the work.

[1] In the earlier paper (Brown, 1935) this dust was referred to under the name of "Brassisan". Since that date this name has been transferred to another preparation containing a different chloronitrobenzene, namely, trichlorodinitrobenzene, which is of use in controlling club-root disease of Brassicae (Smieton, 1939) but which cannot be used with safety on lettuce. The powder containing pentachloronitrobenzene, known in the earlier paper as "Brassisan", is now termed "Folosan".

It will be convenient to give first the main result which has come from a series of experiments extending over four seasons, and later to outline the factors which contribute to the main problem, and the modifications of treatment which may be made to suit the varying conditions of different seasons.

In the first two seasons the variety "Trocadero" was used; in later seasons the variety "Trocadero Improved".

Effect of fungicidal treatment on the stand of seedlings in frames, on percentage survival in the field and on date of maturity

Table 1 gives the results, in simplified form, of experiments carried out over four seasons with respect to the two particulars first stated: the variations, from one year to another, in times of sowing and transplanting are shown. In each year except the first, when special precautions were not taken, exactly equal weights of seed were sown in all frames. A certain number of these served as controls, receiving no fungicidal treatment; the remainder were treated a varying number of times—twice at least and at most six times—between the date of sowing and transplanting. In general, sowings made at or before mid-October were treated 3–4 times, and later sowings 2–3 times. The data of stand of seedlings represent the averages of duplicate sets of frames, except for the treated frames of season 1938–9 which were run in quadruplicate. The number of seedlings planted out in the field varied somewhat from year to year, in some cases being limited by the relatively small number available in the control frames. In the seasons 1937–8 and 1938–9 a high degree of replication was arranged in the plantings, so that the statistical significance of the field data for these years can be assessed. No such measure of significance is obtainable for the earlier years, but the conclusions to be drawn are sufficiently obvious.

Table 1. *Effect of fungicidal treatment on stand of seedlings in frame and on survival in the field*

			Control		Treated	
Year	Date of sowing	Date of planting	Stand of plants per frame	% survival in field	Stand of plants per frame	% survival in field
1935–6	16 Oct.	4–11 Mar.	445	15·5	2550	75
1936–7	6 Oct.	2–5 Mar.	106	15	1128	59
	16 Oct.	2–5 Mar.	216	43	1591	58
	4 Nov.	2–5 Mar.	828	42	1947	64
1937–8	11 Oct.	7 Mar.	290	50	1058	86
	26 Oct.	17 Mar.	1558	55	1763	80
1938–9	13 Oct.*	19 Jan.	163	37	902	85
	27 Oct.	23 Feb.	224	40	1756	66·5

* The seedlings in these frames were uniformly thinned 5 weeks after sowing.

In the earlier years, plantings in the field were made at half the normal spacing. This was for economy of ground and because attention was directed mainly to survival of the plants. Records of survival extended to within 2–3 weeks of the date of hearting. Occasional losses occurred up to the time of maturity, but the figures recorded give a close approximation to the number of plants which matured. As the plants had by that time become very crowded, i.e. in plots where the survival was good, no attempt was made to determine accurately the effect of treatments upon date of maturity. In the seasons 1937–8 and 1938–9 the field plots were extended, and the plants were set out at commercial spacing. In these years the records

of survival were continued to the date at which cutting began. A small proportion of plants in all plots do not heart out (chiefly on account of sporadic mosaic disease): these are included in the figures given, though such plants were not cut.

An outstanding result shown in Table 1 is the consistently great increase in stand of seedlings obtained by fungicidal treatment. The numbers per frame, in the treated lots, were in fact often too great for practical purposes, a result of too high a rate of seeding. On the other hand, the stand of plants in untreated frames was often very poor, representing in some cases merely $\frac{1}{5}$–$\frac{1}{10}$ of what would be reckoned a satisfactory crop. It is thus clear that the development of disease within the frames can largely be held in check by dusting treatment, under conditions which, as shown by the behaviour of the controls, are highly favourable to it (Pl. 21, fig. 1). Inspection of the data for successive sowings made in 1936–7 and 1937–8 shows that the incidence of disease in the frames is much accentuated by early sowing, a conclusion which is in agreement with the experience of growers.

The effect of dusting treatments on survival of seedlings in the field was invariably beneficial, in some plantings very markedly so (Pl. 21, figs. 2–4). The degree of improvement was, however, unequal in the different experiments: e.g. the very good results obtained in the plantings of seasons 1935–6 and 1937–8 are in contrast with some rather poor results in 1936–7. Variant factors—e.g. details of dusting procedure such as amounts, numbers and dates of application, in the different years; conditions of soil and weather at and after transplanting, etc.—will be considered later.

The effect of various modifications of the fungicidal treatment on date of maturity were assessed for the seasons 1937–8 and 1938–9. According to the treatment given the average date of maturity was postponed by 0–7 days, i.e. in some experiments maturity was not delayed, whereas in others there was a delay which would probably be of material significance from a grower's point of view. Recommendations for obtaining the maximum of disease control with minimum check to development will be outlined later.

Damping-off and mildew

The loss of plants which occurs in frames, and later in the field, is usually ascribed to *Botrytis*, but it has become clear that a number of fungi are concerned. At the moment one can assign a share to damping-off fungi and to the mildew fungus (*Bremia*), but whereas each of these can be recognized fairly readily when occurring singly, they are liable to occur together, in which case a separation of symptoms and a determination of the primary cause of disease are difficult if not impossible. This applies particularly to the distinction between *Botrytis* disease and damping-off. Numerous data have been accumulated on the subject of the latter disease (which also is caused by several organisms), and these will be published in a later paper. Here it is sufficient to give the results in outline, so as to indicate the general relation of damping-off and mildew to the *Botrytis* problem.

Damping-off. With reasonable care, there is in general no difficulty in obtaining satisfactory emergence of lettuce seed. Experiments with particular soils have, however, shown that, even with conditions of soil moisture and temperature suitable for good germination, low emergence (e.g. 30–40% of good seed sown) has resulted. That such low emergence is caused by parasitic action is shown by the substantial improvement obtained by fungicidal

treatment, in one experiment from 39 to 92% by use of formalin powder. The Pcnb preparation used in this work is ineffective in this connection. So far as investigations have gone, the causal agents of pre-emergence damping-off are species of *Pythium*. From time to time poor and patchy stands of young seedlings have been seen on growers' premises, but it is not certain how far the trouble was due to inadequate preparation of the seed bed, e.g. the presence of dry patches. In general, however, damping-off of this type is relatively unimportant, stands of seedlings frequently being much too thick to allow of good growth.

Typical damping-off is seen in the development of spreading patches of dead plants, beginning sporadically in the frames and much favoured by the presence of drips. The fungus primarily concerned is *Rhizoctonia Solani*, as is shown by the fact that isolations from plants which have damped off at growers' premises have almost invariably yielded this fungus. At Slough other forms of damping-off occur but here also *R. Solani* is the dominant fungus. Plants are attacked at or a little below ground level, the stem becoming brown and constricted: young seedlings so affected soon fall over and die.

The damage produced in the seed bed by *R. Solani* may be in the first instance a material loss of plants, since the patches of dead plants are often numerous and each one may spread to a large size. Later, the mass of dead seedlings usually develops *Botrytis* spores in abundance and thus a large quantity of inoculum is made available for the development of *Botrytis* attack. There is little doubt that most if not all of the so-called *Botrytis* disease in young seedlings (i.e. in November and December of most seasons) is really damping-off. Later (usually in February or March but as early as December in some seasons) typical *Botrytis* disease may be rampant but it is highly probable that *Rhizoctonia* is still an important contributory to the loss involved, and when the plants are set out in the field it is possible that some of the loss which is apparently caused by *Botrytis*, to judge by the abundant development of *Botrytis* spores on the affected plants, arises in part from damping-off. It may be noted that American workers (Townsend, 1934) attach great importance to *Rhizoctonia Solani* as an agent causing the death of large plants in the field.

Work in progress has shown that several species of *Pythium* cause types of post-emergence damping-off. One of these produces rotting of the roots with subsequent wilting of the above-ground parts; another gives symptoms which are not easily distinguishable from those due to *Rhizoctonia*. So far as is known damping-off caused by *Pythium* does not spread rapidly from plant to plant, apart from the risk that affected dead plants may come into contact with healthy ones, in which case direct transmission takes place. Its existence should, however, be noted as also the possibility that it may be more prevalent at other times or places, especially since the treatment with Pcnb which effectively controls *Rhizoctonia* is valueless for the control of *Pythium*. It should also be stressed that even isolated damped-off seedlings provide a substrate for the development of *Botrytis* under suitable conditions.

So long as the seedlings carry healthy green cotyledons and are otherwise undamaged, they do not appear to be susceptible to *Botrytis* attack, apart from the chance that they may become infected by contact with dead material infested with *Botrytis*. Even when young seedlings are sprayed with a suspension of *Botrytis* spores the usual effect is the production of a number of minute lesions which dry up and form small dark coloured spots (Stevenson, 1939).

Botrytis spores may develop upon the seed coats, an effect often seen in the course of

germination tests in the laboratory. This has not been noted in frames, but there is no reason to doubt the possibility of its occurrence, and as the seed coats are often carried up in germination *Botrytis* spores may be present in the frame from the earliest stages. There is also the possibility that the unexpanded cotyledons may be infected from contaminated seed coats (Stevenson, 1939) but little practical significance can be attached to this.

Typical *Botrytis* disease is liable to occur when the plants reach a certain stage of development. If the cotyledons and often also the lower leaves become yellow and withered, these moribund tissues are readily invaded by *Botrytis* (Brooks, 1908; Abdel-Salam, 1934). Characteristically the fungus enters the stem at such leaf-bases where it produces a reddish sharply defined lesion ("red-leg") which may quickly eat through the stem, subsequently invading and killing the whole plant. Such conditions tend to occur when the plants become crowded towards planting-out time, and any dying back of the leaves due to mildew or severe frost injury is a contributory factor. Under conditions favourable to *Botrytis* the disease may spread rapidly so that large areas collapse entirely. On the other hand, a frame may appear superficially to be healthy, but a closer examination shows the presence of disease. There may be a scattering of dead plants, with a varying distribution of plants showing lesions which partially girdle the stem. It is from contaminated frames such as these that the survival after planting is likely to be low.

Mildew (*Bremia Lactucae*). This may appear sporadically in the frames from an early stage, often being seen on one or both of the cotyledons within a few weeks of germination. As the fungus sporulates first on the under sides of leaves the degree of infestation within a crop is liable to be underestimated, at least in the earlier stages. Later the effect may be obvious, but the symptoms may become confused if *Botrytis* is also sporulating on the leaves already killed by mildew. The conditions which determine the rate of spread of mildew or its intensity at any time have not been adequately studied. Though this disease may become diffused through the whole stand of plants it frequently develops in a patch-like manner during the winter. If the attack occurs on very young plants, or if there is severe loss of leaves, there is a material check and the plants are likely to be undersized at the time of transplanting. Should the disease not appear until later in the season the stunting effect is less noticeable, but the spread may be rapid. In some seasons the damage caused by mildew is not serious; in others, though the plants may suffer a check, they are said by growers "to grow away from it", i.e. though probably delayed, they finally yield a good crop of plants. In the winter of 1938-9 mildew undoubtedly caused serious damage. It was prevalent (see p. 495) to an unusual degree, and after the severe frost of late December 1938 it was very striking that frames and parts of frames, which had shown heavy mildew infestation before the frost, suffered the greatest damage, large numbers of plants being killed outright. Apart from this extreme effect, mildew is potentially important as furnishing dead leaf tissue which is liable to act as a breeding ground for *Botrytis*.

Variations in mode of treatment and their effects

(1) *Soil treatment at the time of sowing*. Experiments showed that the incorporation of Pcnb in the soil used for covering the seed, or even a dusting of the surface of the soil after the seed had been sown and covered, gave an effective control of *Rhizoctonia* damping-off (Table 2). The first five experiments were carried out with pots or boxes in a greenhouse, the last one with boxes in a cold frame and with the same batch of soil as in Exp. I. The

soil used was known to contain a heavy natural infestation of *Rhizoctonia*. Table 2 gives the number of seedlings which emerged and the percentage of these which subsequently damped off.

TABLE 2. *Control of* Rhizoctonia *damping-off by Pcnb*

	Control		Pcnb in covering soil		Pcnb dusted on surface	
Exp.	No. emerged	Damped off %	No. emerged	Damped off %	No. emerged	Damped off %
I	912	91	919	3	—	—
II	764	90	683	1·7	—	—
III	435	35	426	5	424	9
IV	383	44	390	3	349	3
V	359	65	—	—	369	1
VI	654	93	—	—	650	13

The fungicide was added to the covering soil at the rate of 24–60 oz./c.yd. (treatment of the whole soil of the seed box being unnecessary) and a further experiment showed that the dose could be reduced to 12 oz. Under cold-frame conditions, however, there is the likelihood of a check. For ease of manipulation on a large scale it was considered that the surface dusting method should be investigated, and experiments, some at growers' premises, were carried out over several years. The evidence obtained suggests that, although a check was not invariably produced, the risk of it is sufficient to prevent the method being recommended unless there is strong reason to expect the occurrence of damping-off.

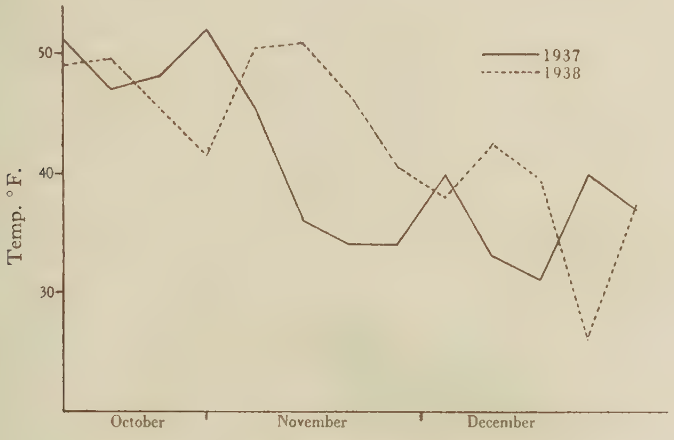
(2) *Dusting treatments following emergence.* These were varied in respect of number, date of application and dosage. Experiments with different filler constituents were carried out in two seasons, and these are described separately.

Apart from a treatment which might be given at the time of sowing, no further applications were made while the seedlings were small, unless such were necessary to check the incidence of damping-off. Experience has shown that visible check to growth is greater the smaller the plants, and it is obvious that a check produced in the earlier part of winter (e.g. November), when the soil as a rule is warm enough to allow of steady growth, is more liable to persist through the cold conditions of mid-winter, when growth may be very slow. The frames were therefore watched for the first signs of damping-off patches, and if any appeared a light dusting was applied throughout. Dead leaves or plants bearing *Botrytis* spores were generally to be found by the latter part of December, especially in the earlier sowings. The frames were then dusted. Further treatments were as a rule given at 3–4 weeks' intervals and a final one immediately before the plants were pulled. The successive treatments have the effect of preserving the stand of plants (Table 1), but against this is to be set a varying degree of check to growth.

Though it is not possible to state what is the best dose for application, the following particulars will serve as guide. In the main experiments with frames, the dosage has in late years been measured by weight, and has generally been at the rate of $\frac{1}{4}$ oz./sq. yd. Replicated experiments with seed boxes in cold frames showed that three applications at this rate give adequate disease control over the 4–5 months' period during which the seedlings are in the frames. A dose of $\frac{1}{8}$ oz./sq. yd. gives a deposit which is visible over the plants and on the soil, whether the filler constituent of the dust be talc or lime, but with the latter a given amount of fungicide, being more bulky, is more readily visible. When the dust

is applied to plants in opened frames, a certain amount is lost, and in this case a useful criterion is the presence of a "just-visible" deposit upon the plants. A dose of $\frac{1}{4}$ oz./sq. yd. represents about 2 lb. per 100 Dutch lights.

(3) *Treatments as affecting growth and maturing of plants.* In the seasons 1937–8 and 1938–9 attention was paid to the effect of various treatments upon the growth of the plants and upon the date of maturing. Further variants were the dates of sowing and of transplanting to the field. Subsidiary experiments were also carried out over a number of seasons with boxes in cold frames. As the results illustrate the effect of a considerable range of conditions, they are given somewhat fully.



Mean weekly temperature

Text-fig. 1.

Text-fig. 1 shows the variation of mean weekly temperature in the screen at Slough during October–December in the two seasons. If one considers the period from early October to the beginning of January, season 1938–9 shows a distinctly higher average temperature over most of November and December. In 1937–8 temperature was fairly high from the date of earliest sowing (11 Oct.) to about 1 Nov., after which it fell rapidly and except for a short period in early December remained low until the end of the year. Thus seedlings of the later sowing (26 Oct.) had barely germinated before the soil temperature became low. In accordance with this, growth of the plants was distinctly backward as compared with normal. In 1938–9, after a short cold period at the beginning of November, temperature became abnormally high later in the month, and though gradually falling it remained favourable for growth until the last week of December when a sudden and severe frost took place. The general growth of the plants was therefore so advanced that in the latter part of December the earlier sowings had nearly reached a size suitable for transplanting to the field. In this season mildew began early and became abnormally prevalent, and many of the mildewed plants succumbed or were severely damaged by the sudden and severe frost which followed.

In both seasons two preparations of the fungicide were compared, one with talc filler and the other with slaked lime. A surface dusting of the soil was given immediately after sowing to all frames except the controls and two others marked * in Table 4. In the first year the dose given was $\frac{1}{2}$ oz./sq. yd. and subsequent dustings were only roughly quantitative. In the second year the initial dose was reduced by one-half, and in the later dustings weighed amounts of the fungicides were blown into the frames.

A check to growth, which persisted in varying degree during the winter, was produced by the soil

treatment. This was slight in the first sowing of the warm autumn of 1938–9. In the colder pre-Christmas period of 1937–8 it was very marked, and with the additional effect of later dustings was obvious at planting time, especially where talc was the filler.

In the season 1938–9 the relationship between type of filler and development of mildew was distinct. Mildew began early in the first sowing and was particularly prominent in the frames which had had the lime preparation. After the frost the control frames were ravaged by *Botrytis* which had appeared earlier; the frames treated with Pcnb (lime) showed obvious damage in that the leaves and whole plants had been destroyed by the combined effect of frost and mildew; the frames treated with Pcnb (talc), though suffering to some extent, maintained their stand of plants. In the second sowing little mildew was present before the frost, but later the intensifying effect of the lime preparation on mildew was again shown.

Plantings from the frames were made in randomized blocks. The method of determining the average day of maturity will be made clear by an example. Suppose that a plants are cut at the first date of cutting, and that b, c, d, etc., are cut at successive 3-day intervals, the average day of maturing is given by

$$a \times 0 + b \times 3 + c \times 6 + d \times 9 \ldots$$

divided by the total number of plants. Alternatively, the effect of treatments on maturing of the crop was assessed by determining the date at which one-half or three-quarters of the crop had matured. The results were substantially the same whichever method of assessment was adopted (Tables 3 and 4).

TABLE 3. *Effect of treatments on survival of plants and on date of maturity in season* 1937–8

Description	Treatment in frame	Stand in frame	% survival in field	Av. day of maturing
Exp. I. Sown 11. x. 37. Planted, 7–8. iii. 38, in 17 replications of 12 plants	Control	430	37	9·5
	Control	152	—	—
	Dusted, talc filler	858	76	17·0
	Dusted, lime filler	1259	93	10·3
Exp. II. Sown 26. x. 37. Planted, 17. iii. 38, in 14 replications of 12 plants	Control	1524	54	15·3
	Control	1593	57	15·5
	Control, final dusting only, talc filler	—	83	15·5
	Control, final dusting only, lime filler	—	86	14·4
	Dusted, talc filler	1849	73	20·1
	Dusted, lime filler	1677	88	16·2
Statistical data:				
Exp. I. $n=30$	Difference between means for $P=0{\cdot}05$	—	8·89	2·14
Exp. II. $n=26$	Difference between means for $P=0{\cdot}5$	—	12·0	2·5

The following points emerge from Table 3.

(1) Both treatments gave a marked increase in the stand of plants in the early-sown frames; with the later sowing there was a strong stand in the control frames, and therefore the improvement, though shown, was much less.

(2) Both treatments gave highly significant increases in survival as compared with the controls. In two of the three comparisons available, the lime preparation gave statistically significant improvement in survival as compared with the talc preparation.

(3) The frames treated with the talc fungicide throughout the winter gave crops which matured significantly later than the controls (7–8 days in Exp. I and 4–5 days in Exp. II). The delay to maturity caused by the lime fungicide was about 1 day, which is non-significant.

(4) A striking improvement in survival in the field was obtained by a single treatment given immediately before transplanting. The survival data are of the order of those obtained by repeated dustings and there is no suggestion of a check to maturity. Where, therefore, a satisfactory stand of plants has been obtained in frames without any fungicidal treatment, a great improvement in field survival may be obtained in the simple manner indicated.

TABLE 4. *Effect of treatments on survival of plants and on date of maturity in season* 1938–9

Description	Treatment	Stand in frame	% survival in field	Av. day of maturing
Exp. I. Sown 13. x. 38. Planted, 19. i. 39, in 9 replications of 12 plants. (All frames of this sowing thinned)	Control	144	30	6·2
	Control	182	44	4·4
	Dusted, talc filler	1057	77	7·1
	Dusted, talc filler	1097	85	6·4
	Dusted, lime filler	848	89	7·2
	Dusted, lime filler	608	88	6·1
Exp. II*a*. Sown 13. x. 38. Planted, 23. ii. 39, in 17 replications of 12 plants	Dusted, talc filler	As above	57	11·2
	Dusted, talc filler	As above	60	12·7
	Dusted, lime filler	As above	81	10·4
	Dusted, lime filler	As above	49	13·5
Exp. II*b*. Sown 27. x. 38. Planted, 24. ii. 39, in 17 replications of 12 plants	Control	335	40	11·8
	Control	113	—	—
	Dusted, talc filler	2007	67	13·0
	Dusted, talc filler*	1884	63	12·3
	Dusted, lime filler	1406	56	16·9
	Dusted, lime filler*	1730	80	13·2
Statistical data:				
Exp. I. $n=16$	Difference between means for $P=0·05$	—	14·6	2·0
Exp. II. $n=30$	Difference between means for $P=0·05$	—	11·3	1·5

* No treatment at time of sowing.

A further planting (7 Mar.) of the later sowing gave results closely similar to those of Exp. II*b*. The following results are shown in Table 4.

(1) Both treatments gave large increases in the stand of plants in the frames, talc proving to be a superior filler to lime, especially in the earlier sowing.

(2) In the early planting (Exp. I) where the best available plants were chosen from the frames there was a considerable increase in survival by both treatments. In the later plantings (Exp. II*a* and II*b*) two frames treated with Pcnb (lime) gave particularly good results, while two which were observed to be specially checked were distinctly inferior; all four frames treated with Pcnb (talc) gave an intermediate result.

(3) In the early planting neither treatment caused any check to maturity (unless the second control be considered alone). In the second planting (Exp. II*b*), treatment with Pcnb (talc) gave no significant retardation of maturity as against a significant delay of 5 days in the case of one of the frames treated with Pcnb (lime). This was the frame in which check to growth had been most marked and the same observation applies to one of the frames treated with Pcnb (lime) in Exp. II*a*.

As the various replicated plots of Tables 3 and 4 were not always in the same set as between one experiment and another (though all were close together in the field), a strict comparison between the several experiments is not valid. However, the following broad conclusions, supported by another experiment (not quoted) in which a strict comparison was possible, can be drawn. In both years the effect of early sowing and planting was to advance maturity by about 6 days as compared with later sowing and planting. Comparison of similar treatments in Table 4 (Exps. I and II*a*) suggests that most of this advantage is lost unless early sowing is followed by early planting. No emphasis need be placed on the actual magnitude of the figures, as the date of maturing of various plantings would obviously be affected by the weather conditions of the particular season. The period of cutting (May–June) of 1939 coincided with a warm spell which tended to even up the various plantings in respect of maturity. In both years the average weight of various batches was determined, and in no case was any effect, such as reduction in size of plant, noted as a result of any of the treatments.

The results of Tables 3 and 4 indicate that where there is a marked check to the plants which persists up to the time of transplanting, there is likely to be a delay in date of maturing. In 1937–8 this occurred in the frames treated with Pcnb with talc filler; in 1938–9 in two frames treated with the lime modification. In the latter the cause was considered to be a check accentuated by mildew which appeared to be favoured by Pcnb with lime filler. When this check was not serious the fungicide with lime gave the better survival in the field. These indications are borne out by five subsidiary experiments in which more check to growth and more bleaching of the foliage were caused by dusting with Pcnb (talc) than with the same weight of Pcnb (lime).

As regards control of mildew, there is strong evidence that the replacement of talc by lime as filler of Pcnb or of cuprous oxide materially reduces fungicidal efficiency. This is given in Table 5 where the data represent percentage infection of plants and are derived from replicated experiments. With one exception the individual differences recorded, which are all in favour of the talc filler, are significant in themselves.

TABLE 5. *Percentage plants infected with mildew as related to filler of fungicide*

Fungicide	Lime filler	Talc filler
Cuprous oxide	96	25
Cuprous oxide	100	80
Pcnb	80	66
Pcnb	99	31

Cuprous oxide, though giving good control of mildew may cause severe leaf scorch. How far Pcnb (talc) is usable as a means of partial control is uncertain: at the moment it appears to be more effective when used early to prevent an outbreak than when used later to check the spread of established mildew. Over and above the distinct effect which lime exercises in diminishing the fungicidal control of mildew (see Table 5), there is evidence that the presence of lime alone on lettuce foliage increases susceptibility to mildew. Thus in an experiment four boxes of seedlings were dusted with lime, four others being kept as controls. All were then equally sprayed with mildew spores. The percentage of infected plants was 42 in the dusted boxes as against 19·2 in the controls. This represented a significant difference.

ADDITIONAL EXPERIMENTS

At various times the following varieties have been used to some extent either at Slough or at growers' premises: Lobjoit's Dark Green Cos, May Queen, May King, Feltham King, Market Favourite, Sutton's Imperial, Cheshunt Early Giant and Early French Frame. Out of ten dusting trials in the three seasons 1935–8 at growers' farms, seven showed good control of disease in the frames; in the remainder no appreciable disease was present. In two planting-out experiments where the results could be followed, very satisfactory results were obtained.[1]

DISCUSSION AND RECOMMENDATIONS

The practical object of sowing lettuce in cold frames in October–November, maintaining the plants at considerable expense of labour during the winter months, and planting out in early spring is to enable a grower to cut for market some weeks ahead of crops which have been drilled in the open ground in March or April. The gain in time may amount to only a few weeks but the gain in value is often considerable, especially when a spell of warm weather in May or early June causes a sudden demand for salads before the spring-sown crop is available. Aiming at earlier maturity, growers have experimented with earlier and earlier sowings, often with disastrous results from fungal trouble. The general practice near London is to begin sowing about 25 Oct., and to make successional sowings up to about mid-November. Some growers have even abandoned sowings in the late autumn and sow in cold frames or on the floors of cold or slightly heated greenhouses in January. Late sowing gives a measurable control of disease and in some years an adequate control, but, even so, considerable damage occurs in the frames in some years at some farms and in most years at other farms.

[1] *Added Note* (June 1940). In a further trial during the current season, a six-fold replicated planting of treated and untreated Feltham King and Market Favourite at a grower's farm gave the following survival data at the time when cutting was about to begin:

	Treated %	Untreated %
Feltham King	94	47·6
Market Favourite	93	42·5

There was no obvious indication of any check to maturity caused by treatment.

The desirability of early sowing, with a view to early planting and therefore early maturity, varies from farm to farm. Certain growers have land of sufficiently light texture that planting is feasible in most years in February or even in January. Their problem is to obtain by this date seedlings large enough for transplanting, and this can be accomplished only by early sowing. Other growers, with rather heavy land, are generally unable to prepare the ground for planting until well on into March. They are, therefore, often faced with the difficulty that the plants meanwhile are becoming large and spindly, the leaves come into contact with the glass, thereby interfering with opening of the frames after frosty nights, and the mass of plants are in a condition highly favourable to attack by *Botrytis*. In these circumstances, early sowing is to be avoided and the grower must reconcile himself to losing the early market. The use to which the particular grower could put the information contained in this paper would thus depend to a large extent on the conditions prevailing on his farm.

The results obtained in this work show that, by a technique of dusting, strong stands of plants can be maintained in frames when untreated crops more or less fail. The stands of treated plants are much freer of incipient fungal lesions, especially in the neighbourhood of the collar, and the survival in the field of such treated plants is much enhanced. Against these advantages may have to be set two drawbacks.

The first of these is a check to growth of the seedlings by application of the fungicidal dust. The conditions which govern the amount of check produced are now fairly well known viz. check is greater the smaller the plants, the lower the temperature, and the heavier the dose applied. Thus a certain dosage, given in November when the seedlings are small and when ground temperature is low and is likely to become lower, may produce a persistent check, whereas the same treatment in February, when the plants are larger and ground temperature is normally tending to rise, may give no visible check at any time or at most an evanescent effect. In various experiments, the amount of check, measured by the date of maturing of the plants, varied from nil to about 7 days. The latter may represent a very significant amount from the grower's point of view, especially if his aim is to cut over the period when prices are liable to drop sharply.

The second drawback is in relation to frost damage. After the sharp frost in 1938–9, there was a suspicion that the dusted plants suffered more than the undusted, but there were considerable complications arising from mildew (see pp. 495–6) so that a definite conclusion could not be drawn. In the long run, whatever the frost damage might have been, the stand of plants in the dusted frames far exceeded that in the undusted (Table 4). In the current season (Feb. 1940) the effect has been more pronounced and clear-cut. After severe frost, it has become obvious that, even in the virtual absence of mildew, the dusted plants show more injury than do the controls.[1]

[1] *Added Note* (June, 1940). The long frost of Jan.–Feb. 1940 had the effect of causing maximum fungicidal injury and at the same time of keeping fungal attack in abeyance. Hence, when the thaw came, some of the control frames had a better appearance than the dusted, and it was thought that the usual result, viz. a much increased stand of plants by dusting, would not occur. However, *Botrytis* became very active in the control frames and the following results were obtained:

(*a*) Control.	Av. of 3 frames	703 plants
(*b*) Dusted 6 Feb.	Av. of 3 frames	1135 plants
(*c*) Dusted 2 Dec.	Av. of 3 frames	1105 plants
(*d*) Dusted 2 Dec. and 6 Feb.	Av. of 3 frames	942 plants

There was a very marked check in (*d*) at planting-out time, and to a less extent in (*c*), associated with a higher percentage of small plants than in (*a*) or (*b*). Finally, the effects of dusting on date of maturity were: (*b*) no delay, (*c*) 1 day's delay, (*d*) 3 days' delay.

Though the stand of plants in the field has been consistently improved by the dusting method, there has been considerable variation in the amount of improvement shown (Table 1). The reasons for this variation cannot be definitely stated. To some extent, no doubt, the conditions prevailing in the field after planting have an effect, but a study of the records available rather indicates that insufficient dusting was mainly responsible for the inadequate control of the disease in some years. On the basis of experience extending over a considerable number of seasons, the following general procedure may be recommended with confidence for the treatment of spring lettuce.

The application of the fungicide at the time of sowing either by pricking into the top layer of soil at the rate of 1 oz./sq. yd., or more simply by lightly dusting the surface after the seed has been covered controls *Rhizoctonia* damping-off. There is, however, a risk of checking the growth of the young plants (which can be met by advancing the date of sowing by a few days), and this check is more pronounced the colder the subsequent weather. Such treatment at sowing time is, therefore, not recommended unless serious losses have occurred in previous years from damping-off. It would be safer to wait until damping-off begins when a light dusting should be given; or if sporadic bare patches appear, the dusting should be confined to their margins to prevent any further spread. Also, a light dusting should be given if mildew occurs since the treatment gives a partial control of this.

Unless the early winter (November–December) is very mild, no treatments other than the above should be required before the end of the year. When the plants have reached such a size that they are becoming crowded, and the cotyledons and perhaps also the lower leaves are becoming yellow, they have reached a stage at which they are highly susceptible to *Botrytis* attack, and if also, as is usual, occasional damped-off plants lying on the soil show *Botrytis* spores, the frames should be dusted—lightly if the plants are backward, i.e. if the season has been cold, and conversely. During severe frost, dusting should be withheld and the frames should receive good protection. Moderate dustings ($\frac{1}{4}$ oz./sq. yd.) at 3–4 week intervals should be given from this period onwards. As the dust, when present, prevents the sporulation of *Botrytis*, the appearance of spores on any debris or plants within the frame indicates that a further treatment is required. Finally, a thorough dusting should be given immediately before the plants are pulled for transplanting. As the dust adheres to the foliage much better when the latter is wet, dusting should be carried out when the plants are wet with dew, or the plants may be lightly sprayed.

So long as the plants are not too large and crowded an effective treatment is to raise a corner of the frame and blow in the dust, care being taken to distribute it evenly. Later, and especially at the final treatment, it is important that the dust should reach the lower leaves and collars of the seedlings. The frames should then be traversed with the blower and the dust blown vigorously among the plants. Experiments have clearly shown that the final dusting gives a better stand in the field if it is done thoroughly, and that the risk of checking plant growth by such treatment is slight.

Altogether the number of treatments should not in general exceed three or four and most of these would be over the period from the end of December to February or March. If the plants are in forward condition as the result of a mild winter, and especially if outdoor conditions delay transplanting, further treatments would be required. Under these conditions the risk of causing a check to growth is negligible. If, however, the plants are backward on account of a cold winter, treatment should be sparing and it would in general be less called for as there would be less crowding of the plants. After a period of severe frost,

Fig. 1

Fig. 2

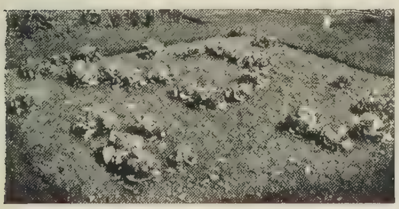


Fig. 3

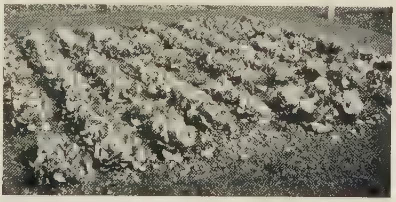


Fig. 4

SMIETON AND BROWN.—*Botrytis* Disease of Lettuce, its Relation to Damping-off

Botrytis is liable to develop quickly on any parts which have been so damaged and, as was well shown in 1940, a treatment immediately following the thaw gives a pronounced benefit.

Summary

1. In experiments carried out over 6 years [including 2 years' work previously described by Brown (1935)], treatments with a dust containing pentachloronitrobenzene applied to lettuce seedlings overwintering in frames consistently gave large increases in the stand of plants available in the spring, and also substantial improvements in the survival of plants when set out in the field. Against this beneficial result is to be set a possible check to the maturing of the plants which varied from nil to about 7 days, according to the precise treatment given.

2. A sketch is given of the relation of various types of damping-off disease, of mildew and of *Botrytis* disease to the problem of carrying crops of lettuce seedlings over winter in cold frames.

3. The efficiency of the fungicide depends to some extent on the nature of the filler constituent. In particular it is shown that, whereas the fungicide containing talc as filler gives some control of mildew, no control is obtained when talc is replaced by lime.

4. General recommendations are given for the carrying out of the dusting programme. Since it is important to avoid checking the growth of seedlings when very young, early dustings, if required to check damping-off or mildew, should be light: later, and especially in seasons when the plants are "forward", heavier applications may be made. In general, three or four dustings should suffice, most of these being applied over the period January–March. The final treatment, given just before the seedlings are pulled for transplanting, should be thorough.

5. The fungicide tends to increase susceptibility of the plants to frost injury. If, therefore, a severe frost occurs after the plants have been dusted, a certain amount of leaf scorch may appear. This effect was shown distinctly in only one of the seasons covered by the experiments (in 1939–40); in most winters it was not perceptible.

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REFERENCES

Abdel-Salam, M. M. (1934). *Botrytis* disease of lettuce. *J. Pomol.* **12**, 15.

Brooks, F. T. (1908). Observations on the biology of *Botrytis cinerea*. *Ann. Bot., Lond.*, **22**, 479.

Brown, W. (1935). On the *Botrytis* disease of lettuce with special reference to its control. *J. Pomol.* **13**, 247.

Smieton, M. J. (1939). On the use of chlorinated nitrobenzenes for the control of club root disease of Brassicae. *J. Pomol.* **17**, 195.

Stevenson, G. (1939). On the occurrence and spread of the ring spot disease of lettuce caused by *Marssonina panattoniana*. *J. Pomol.* **17**, 27.

Townsend, G. R. (1934). Bottom rot of lettuce. *Mem. Cornell agric. exp. Sta.* no. 158.

EXPLANATION OF PLATE 21

Fig. 1. On right, undusted; on left, dusted (three treatments); frame sown on 16 October.

Fig. 2. Part of replicated series of 1-row plots; rows 1, 3, 4, 5 (from left) had various modifications of treatment; row 2, untreated.

Fig. 3. Plot in which were planted 120 seedlings, free from any obvious collar lesion and taken from undusted frames.

Fig. 4. As in fig. 3, but a single thorough dusting given immediately before transplanting.

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BACTERIUM RHAPONTICUM (MILLARD) DOWSON, A CAUSE OF CROWN-ROT DISEASE OF RHUBARB

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Millard (1924) described a crown-rot of rhubarb (*Rheum rhaponticum*), prevalent in the rhubarb-growing areas around Leeds, and caused by a bacterium which he named *Bacterium rhaponticum*. White (1936) studied the rhubarb disease and isolated an organism which corresponded with Millard's bacterium, but was unable to secure positive results with inoculations. Johnson (1936) indicated that infection by the eelworm *Anguillulina dipsaci* Kühn is frequently a pre-requisite for the appearance of crown-rot; later (1939) he was inclined to regard the eelworm as a primary cause of crown-rot.

The writer investigated a crown-rot disease of Victoria rhubarb prevalent in cottage gardens in central Yorkshire. The symptoms of this disease agree with those described by Millard. A bacterium was isolated and proved pathogenic; its characters correspond with those of Millard's organism, and the writer is convinced that he is working with the same disease as did Millard.

Millard's description of his organism was not sufficiently extensive, and on the data available it has been placed (Bergey, 1939) in the genus *Phytomonas*. A more complete investigation of the characters of the organism has shown that it should be included in Bergey's genus *Erwinia*. In this account the generic scheme suggested by Dowson (1939) is followed and the rhubarb organism is therefore called *Bacterium rhaponticum*. A fuller description of the organism is given and its relationship with the bacterial "soft-rot" organisms discussed.

Symptoms

The chief feature is the rotting and the death of the terminal or "crown" bud of the *corm*; this is accompanied by a soft, chocolate-coloured rotting of the pith tissues, resulting in the formation of a cavity. The tissues outside the vascular ring of the corm are usually not affected by the internal rotting but later they may wither and die. The death of the terminal bud is followed by the growth of the lateral buds which may grow quite healthily but are often diseased. In these buds the rotting may start from the base, but very often it appears to start in the laminae of the young leaves. Mature leaves are seldom attacked except under very wet conditions, when the leaf-base may be affected. In summer the leaves on diseased plants are often puce-coloured.

Histologically, the bacteria are at first confined to the intercellular spaces and there is little separation of cells, but at a later stage of the disease the cells become separated and may be invaded by bacteria.

Isolation of the pathogen

At the advancing edge of a rotted area, separating the healthy parts from the soft and rotting parts, there is usually a narrow zone of discoloured but firm tissue. The tissues in

this boundary layer are permeated with bacteria but fungi and eelworms are absent. In the completely rotted tissue, however, both are often present. Isolations were made from diseased rhizome and from rotting bud tissue. Portions of the boundary layer were removed aseptically and crushed in sterile water; after a lapse of 10 min. the diffusion was streaked out on bullock-heart-infusion agar. Several plates were streaked in each isolation and altogether eleven isolations have been made.

In each isolation, small numbers of colonies of *Bact. aerogenes* and of a species of *Pseudomonas* were obtained on the isolation plates; these organisms were subsequently proved to be non-pathogenic. Most of the colonies were colourless, reaching 3 mm. diam. after 2 days' incubation at 25° C. Six of these colonies were subcultured from each plate and inoculated into surface-sterilized slices of rhubarb rhizome. About half of the organisms thus tested produced a rapid pith rot. Twelve pathogenic and twelve non-pathogenic cultures were selected for further study. The pathogenic cultures showed similar biochemical properties and corresponded with Millard's organism. The non-pathogenic cultures all had the same biochemical reactions, but these differed in many respects from those of Millard's organism, and this group was not studied further. In addition, isolations of fungi were made by the usual methods. Three fungi (one of them a species of *Penicillium*) were isolated, but as they were proved non-pathogenic they were discarded. A complete investigation of the characters of the pathogenic bacterium was made and these are now given.

Bacterium Rhaponticum (Millard) Dowson

Six cultures were used in determining the characters of the organism; these cultures came from different isolations. No differences between the various cultures were found except that two were chromogenic. Each isolation was made on bullock-heart-infusion agar and the subsequent subcultures were all made on to this agar.

Pathogenicity. Pathogenicity was soon lost in culture, but by subculturing every 3 weeks virulence was maintained.

Morphology. The organism is a Gram-negative rod, is not acid-fast, does not form spores and is usually non-capsulated. Preparations were made from a 24 hr. old growth on bullock-heart-infusion agar and stained with methylene blue, carbol fuchsin, and by Benian's indian ink relief-staining method. Single cells measure about 1·2–1·5 μ by 0·5–0·8 μ (Millard's measurements were greater than this). The organism possesses three to seven peritrichous flagella (usually five) which were stained by Morton's method (see Dowson, 1939). It is not readily motile, but good motile preparations were obtained from a 36 hr. old growth on damp, freshly prepared heart-infusion agar. Diffusions from plant material have never shown motility. Millard did not observe motility, nor record the result of flagellum staining.

Carbohydrate metabolism. In fermentation tests the carbohydrates were dissolved in a solution of inorganic salts according to the method described in the *Manual of Methods*, 1936, **2**, 14, of the Society of American Bacteriologists. Results were recorded after 7 days. Acid but no gas was formed from arabinose, xylose, dextrose, laevulose, galactose, mannose, sucrose, maltose, lactose, mannitol, glycerol and salicin. Starch could not be used as a sole source of carbon and no indication has been obtained that starch is ever attacked.

Litmus milk. Acid is formed in 3 or 4 days, with or without a slight curd separation. There was no clotting, even after several weeks.

Nitrate reduction. Good, long-standing growth in nitrate-peptone water, with well-marked reduction of nitrate to nitrite.

Indol. No indol produced in broth ("Difco" Bacto-Tryptone).

Vosges-Proskauer reaction. Good growth in fumarate medium (O'Meara, 1931) with a positive acetyl-methyl-carbinol reaction.

Ammonia production. Ammonia produced in solutions containing amino acids (tested with Nessler's solution).

Hydrogen sulphide. No H_2S produced (tested with filter paper impregnated with lead acetate).

Gelatine stab. A beaded growth formed to the bottom of the needle stab; there is no liquefaction.

Uschinsky's solution. Heavy growth and a bulky flocculent precipitate, a pellicle and a thick rim.

Cohn's solution. Moderate growth, without deposit or pellicle.

Fermi's solution. Heavy growth, with a bulky pink deposit, a pellicle and a thick rim: the liquid frequently coloured pink.

Citrate solution. Good growth, with no pellicle and only a slight deposit.

Cultural characters. Colonies on bullock-heart-infusion agar are circular, convex, smooth, glistening, translucent, with entire margins, and are 2–3 mm. diam. on the second day when incubated at 25° C.

On rhubarb-extract agar, colonies are similar but slightly larger, often with a yellowish tinge and with auriculate margins.

On agar slants the growth is translucent to whitish, glistening, smooth, slightly butyrous, with entire and raised margins.

In tryptophane broth there is a uniform turbulence with a fragile pellicle, a slight rim and a slight flocculent deposit.

Growth. Growth takes place at all temperatures from 0 to 37° C. (possibly at higher temperatures). When dried by the method described by Brown (1923) the organism withstands drying for at least 8 days and also night temperatures of $-5°$ C.

Chromogenesis. Some strains of the organism, when grown after isolation on autoclaved potato, secreted a pink pigment. These chromogenic strains were as pathogenic as non-chromogenic strains and did not differ in any other respect from the latter. If non-chromogenic strains were subcultured into tryptophane broth and incubated in the refrigerator (3° C.) they usually secreted a pink pigment into the medium; if a subculture was made from these on to potato the resulting culture was chromogenic. When non-chromogenic strains were grown in Uschinsky's solution or in Fermi's solution they became chromogenic and frequently coloured the solution; transfers from these solutions on to autoclaved potato always gave the pink colour. The same result was obtained (with less constancy) when citrate solution was used. The chromogenic powers thus induced were retained after several subsequent normal agar transfers. Passage through Cohn's solution suppressed induced chromogenesis but not natural chromogenesis.

The pigment concerned is entirely extracellular; if any strain of the organism is grown on potato-glucose agar (but not on potato agar) the bacterial growth remains colourless but after about 3 weeks a pink colour has diffused throughout the agar. The conditions governing the production of the pigment, and its chemical nature, are receiving further study.

Inoculation experiments

Although the ability of *Bact. rhaponticum* to attack rhubarb rhizome had already been proved, the following experiments were made.

Series I. A rhubarb crown, growing in a large plant-pot, was inoculated in the following manner. The dead scales around the base of a leaf were removed and the whole of the surface was washed with methylated spirit. The leaf was then torn away and into the sterile surface thus exposed the bacteria from a 24 hr. old agar culture were pricked with a sterile needle. The wound was then covered with sterile vaseline. The plant later developed the typical symptoms of the disease. Another plant was inoculated by pricks on a cut and sterilized surface which was left exposed to the air. No rotting developed, but when the inoculation was repeated and the plant covered by a bell-jar to give a saturated atmosphere, rotting developed and affected the whole crown.

Series II. Buds were removed from healthy rhubarb plants. The protecting leaf-base was surface sterilized, split with a sterile scalpel, and the young leaves inoculated in the lamina or in the petiole. The young tissues could not be satisfactorily sterilized on account of the mucilage which bathes them, so an equal number of wounded controls was kept. No infection could be obtained unless the buds were kept in a fairly saturated atmosphere, but under these conditions a local or an extensive rot was obtained with lamina inoculations and an extensive rot with petiole inoculations.

Series III. For these experiments young but mature leaves were used. After surface sterilization, inoculations were made in the lamina, in the petiole, or in the leaf base. With lamina inoculations no

rotting occurred, or only a very local rot. With petiole inoculations a local non-spreading rot occurred, with large quantities of bacterial exudate. With leaf-base inoculations an extensive rot took place without discoloration of the tissues. In all these experiments, successful results were obtained only in a damp atmosphere. No infection of old mature leaves could be obtained under any conditions.

The organism was re-isolated from the diseased material of series I and II and was again proved pathogenic by inoculation on to surface-sterilized slices of rhubarb rhizome.

These experiments show that *Bact. rhaponticum* can cause a crown-rot and bud-rot of rhubarb. Under experimental conditions a fairly saturated atmosphere is needed for successful infection. Under field conditions the base of the crown is always surrounded by much dead or dying material (old leaves, leaf bases, etc.) and this is invaded by a variety of organisms. For a relatively long period after rain, water is held in the gaps between the sheathing leaf bases and the corm and infection perhaps takes place from this source. Within the sheathing leaf base protecting the bud the young leaves and the growing point are bathed in a mucilaginous substance in which bacteria can multiply abundantly.

The relation of *Anguillulina dipsaci* to the disease

Johnson (1939) points out that the eelworm *Anguillulina dipsaci* is constantly associated with crown-rot disease. In a series of experiments in which "dry infective material" containing *A. dipsaci* was added to the soil he succeeded in reproducing the disease in healthy plants. Johnson does not mention any examination of his material for the presence of *Bact. rhaponticum*. If this was present, the "infective material" contained not only the eelworm but an active pathogen. In none of his experiments were Koch's postulates fulfilled, in that the inoculum used was not a pure culture of the suspected organism but tissue which inevitably contained many other organisms. Moreover, the constant presence of *A. dipsaci* in infected material does not imply that it "is a most serious primary cause of disease", for many organisms (e.g. the secondary and non-pathogenic *Bact. aerogenes*) are almost constantly present in rotted plant tissues but no pathogenic role can usually be ascribed to them.

The writer has observed *A. dipsaci* associated with the rotting in all the naturally infected material examined, but in all the inoculation experiments with a pure culture of *Bact. rhaponticum* (except series I, which was not examined) a study of the typical rot produced revealed the complete absence of *A. dipsaci*. In an attempt to clarify the situation, the following inoculation experiments were performed.

Series IV. Four rhizomes about 2 in. in diameter were used; these were cut off about 3 in. behind the terminal bud and the cut surfaces were sterilized. Sett A was planted in sterilized soil and watered liberally with a suspension of *Bact. rhaponticum*. Sett B was treated in the same way, but, after watering, a needle was driven through the drainage hole of the plant-pot and the sett wounded several times in the pith. Sett C was planted in sterilized soil to which naturally rotted tissue (containing eelworms) had been added, and this soil was watered with distilled water. Sett D, the control, was planted in sterilized soil and watered with distilled water.

After a month the terminal buds of setts B and C were dead, the leaf petioles were rotted at the base and the rhizome tissue was rotted. *Bact. rhaponticum* was isolated from both setts; the rotted tissues of sett C contained eelworms but no eelworms were found in sett B. Setts A and D remained perfectly healthy.

Series V. Surface-sterilized Victoria rhubarb seeds were planted in sterile soil in large test-tubes and were watered with recently-distilled water. The seedlings were divided into four groups. The first group was held as control and grew normally. A suspension of *Bact. rhaponticum* was added to the second and third groups; the roots of the second group were then wounded by needle pricks whilst the plants in the third group were not wounded. To the fourth group was added a suspension of crushed, naturally diseased tissue (containing eelworm).

After 10 days the seedlings in group four were rotting from the base and examination showed the presence of both bacteria and eelworms in their tissues. After 14 days the seedlings in group two were also rotting, but only bacteria were found in the diseased tissues. In group three, one seedling out of four died.

Johnson's experiments, and the experiments of series IV and V, show that infection of healthy plants from the soil usually occurs when *A. dipsaci* is present. In the absence of the eelworm rotting ensues only if the bacterium is introduced into the tissues through a penetrating wound. It is thus probable that *A. dipsaci* can act as an agent of infection, by introducing the pathogen from the soil through the wounds it makes, and possibly aiding the subsequent spread of infection within the plant. It is thus possible that any control of *A. dipsaci* (such as the exclusion from the crop rotation of the alternate host plants described by Johnson) may give some degree of control of the crown-rot disease.

Some other agent may also be involved in the spreading of the disease, however, for on several occasions extensive rotted lesions have been observed associated with "feeding wounds" in the leaf base tissues, and from one such lesion *Bact. rhaponticum* was isolated. No clue as to the identity of this possible vector has been obtained.

Relation between *Bacterium rhaponticum* and the soft-rot bacteria

In its biochemical characters, *Bact. rhaponticum* resembles *Bact. aroideae* (Towns.) Dowson, and the two bacteria were therefore carefully compared. It was found that *Bact. aroideae* turned litmus milk red and produced a firm clot with the separation of the whey. Moreover, *Bact. aroideae* completely liquefied gelatine with the formation of a green precipitate. In these two characters, and in the fact that it is not chromogenic, *Bact. aroideae* differs from *Bact. rhaponticum*.

The action of *Bact. rhaponticum* on a variety of hosts was compared with that of *Bact. aroideae*; for interest, inoculations were made at the same time with *Bact. carotovorum* (Jones) Dowson and *Bact. phytophthorum* (Appel) Dowson.[1]

Series VI. Surface sterilized slices of rhubarb rhizome were inoculated with the four organisms. *Bact. carotovorum* and *Bact. phytophthorum* caused no rotting, or only a localized rot, but *Bact. aroideae* caused an extensive rot of all the tissues of the rhizome, and not merely of the pith as did *Bact. rhaponticum*. Moreover, the *Bact. aroideae* rot was not discoloured whereas the *Bact. rhaponticum* rot is dark brown.

A similar result was obtained when Victoria rhubarb seedlings (growing in cotton wool in sterile Petri dishes) were inoculated. *Bact. rhaponticum* and *Bact. aroideae* caused an

[1] The cultures of *Bact. aroideae*, *Bact. carotovorum* and *Bact. phytophthorum* were kindly supplied by Dr W. J. Dowson, of the Botany School, Cambridge. For the purposes of this paper the distinction drawn between *Bact. carotovorum* and *Bact. phytophthorum* by Dowson (1940) has been accepted.

extensive stem and leaf rot whereas *Bact. carotovorum* and *Bact. phytophthorum* did not attack the young seedlings.

When the three soft-rot bacteria were inoculated into rhubarb buds a rot developed in every case. With *Bact. aroideae* the rot was very extensive and resembled that due to *Bact. rhaponticum*; the other two organisms caused an extensive rotting of the young laminae but only a local rot of the young petioles.

The four organisms were inoculated into the pith of young setts which were subsequently planted. *Bact. rhaponticum* caused an extensive pith rot and *Bact. aroideae* a much more localized one. The rotting due to the other two organisms was very localized.

Series VII. The action of *Bact. rhaponticum* and *Bact. aroideae* (as well as of *Bact. carotovorum* and *Bact. phytophthorum*) on a variety of host tissues was studied. The two latter organisms usually produced similar results except that more discoloration was associated with the *Bact. phytophthorum* rots.

Bact. rhaponticum did not attack raw potato slices (young and old potatoes), carrot slices (young and old carrots), lettuce, cauliflower, cabbage, turnip slices or parsnip slices, whereas *Bact. aroideae* and the other two organisms rotted all these tissues. All four organisms caused a rot of cucumber slices and of onion (on white onion *Bact. rhaponticum* secreted a pink pigment which coloured the rotted tissues). On celery, *Bact. carotovorum* and *Bact. phytophthorum* caused an extensive rot of both young and old leaves whereas *Bact. aroideae* attacked only the young petioles; *Bact. rhaponticum* did not attack young petioles but caused a local rot, with pink colour formation, in old petioles. *Bact. rhaponticum* caused no rotting of young leaves of *Iris germanica* whereas the other three organisms caused an extensive rot (the strain of *Bact. aroideae* studied by Massey (1924) would not rot iris leaves). *Bact. aroideae* caused an extensive rot of the petioles and laminae of the leaves of *Richardia* (the Calla lily) but the other three organisms did not attack this host. On *Arum maculatum*, *Bact. aroideae* caused a rapid and extensive petiole rot, *Bact. carotovorum* and *Bact. phytophthorum* caused a slow and more localized rot, but *Bact. rhaponticum* did not attack this host.

On the basis of the range of host plants used in these experiments *Bact. carotovorum* could not be differentiated from *Bact. phytophthorum*. *Bact. aroideae* differs from *Bact. carotovorum* in the action on *Richardia*, *Arum*, and rhubarb seedlings and rhizome. In the type of rot produced, however, *Bact. aroideae* often differs from *Bact. carotovorum* as the *aroideae* rot is not usually accompanied by much discoloration of the rotted tissues.

The differences in biochemical properties, and the totally different pathogenic ability of the two organisms—one restricted in its powers of attack and one almost omnivorous—leaves no doubt that *Bact. rhaponticum* and *Bact. aroideae* are distinct species; indeed it is obviously impossible to class *Bact. rhaponticum* as a "soft-rot" organism in the sense of the term as used in plant bacteriology.

In all the isolations made from diseased rhubarb no organism was ever obtained which corresponded with *Bact. aroideae* in its action on litmus milk and gelatine or in its host range, and it is therefore concluded that *Bact. rhaponticum* is probably the only bacterium involved in the crown-rot disease of rhubarb.

Summary

It has been shown that *Bacterium rhaponticum* (Millard) Dowson is a cause of the bud and corm rot of rhubarb known as crown-rot disease. A full description of the organism is given. It is suggested that the eelworm *Anguillulina dipsaci* Kühn is not a primary cause of disease but plays the part of an infective agent by introducing the pathogenic bacterium into healthy plants from the soil.

A comparative study has been made of the pathogenic ability of *Bact. rhaponticum* and of three species of "soft-rot" bacteria. *Bact. rhaponticum* does not belong to that class of "soft-rot" organism. Pathogenic differences between *Bact. aroideae* and *Bact. carotovorum* are described.

REFERENCES

Bergey, D. H. (1939). *Manual of Determinative Bacteriology*, 5th ed. Baltimore: The Williams and Wilkins Co.

Brown, N. A. (1923). Bacterial leaf spot of geranium in the eastern United States. *J. agric. Res.* **23**, 361.

Dowson, W. J. (1939). On the systematic position and generic names of the Gram-negative bacterial plant pathogens. *Zbl. Bakt.* Abt. II, **100**, 177.

—— (1940). Identity of the bacterium causing potato blackleg. *Nature, Lond.*, **145**, 263.

Johnson, L. R. (1936). Observations on the occurrence of *Anguillulina dipsaci* (Kühn, 1858) on rhubarb in Yorkshire. *J. Helminth.* **14**, 77.

—— (1939). Further observations on *Anguillulina dipsaci* in rhubarb. *Ann. appl. Biol.* **26**, 739.

Massey, A. B. (1924). A study of *Bacillus aroideae* Townsend the cause of a soft-rot of tomato, and *B. carotovorus* Jones. *Phytopathology*, **14**, 460.

Millard, W. A. (1924). Crown rot of rhubarb. *Bull. Univ. Leeds and Yorks. Coun. Agric. Educ.* no. 134.

O'Meara, R. A. Q. (1931). A simple delicate and rapid method of detecting the formation of acetyl methyl carbinol by bacteria fermenting carbohydrate. *J. Path. Bact.* **34**, 401.

White, H. L. (1936). Rhubarb crown rot and allied diseases. *Rep. exp. Res. Sta. Cheshunt*, p. 54.

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THE ECOLOGY AND POPULATION DYNAMICS OF THE WILD RABBIT (*ORYCTOLAGUS CUNICULUS*)

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(With Plate 22 and 2 Text-figures)

This paper gives the results of a year's field work on populations of the wild rabbit (*Oryctolagus c. cuniculus* (L.)). The experimental work was intended to be carried on over three years, but it has been thought advisable to publish the data already collected, since they cover probably the most important period of the rabbit's life cycle, namely, the breeding season. Few facts have been recorded about the rabbit, though there is much tradition and hearsay. To this extent these results may be regarded as an advance and as forming the groundwork of a fuller and more complete understanding of the ecological and economic status of the rabbit.

General considerations in the ecology of the rabbit

There is practically no European literature dealing with the subject. Niethammer (1937) is the only author so far traced who has experimented with marking wild rabbits: his methods were random ones (ferreting) and few observations were made. More work has been done in America, notably on the snowshoe rabbit (*Lepus americanus*) and on the cottontail rabbit (*Sylvilagus floridanus*), and in the case of the latter live trapping and marking techniques have been elaborated and census taking has reached an efficient level. Unfortunately, these techniques will not apply to the European wild rabbit on account of the difference in its habits.

With the rabbit the main unit of the population is the colony, so that distribution is more analogous to a contour map, where each peak represents a focal point with a high resident population and the slopes from each peak represent the area of influence of each warren. The fact that the distribution of the population is not random means that traps cannot be set out in grids, a valuable procedure, which equalizes the trap efficiency at each of a series of trappings. The only practicable plan was to study a single warren, so that the figures derived from it could be applied to larger or smaller warrens, or to total areas with known amounts of warren upon them.

Technique

(i) *Trapping*

The warren used in the experiments was chosen because it was fairly isolated, the nearest burrow being about 100 yd. away, and because it was in the middle of a grass field and well defined in its boundaries. The actual warren area was about ½ acre in extent (Pl. 22, fig. 1), contained about 104 burrows, and was overgrown with a thick cover of nettles. During the winter of 1938–9 a fence was erected around the warren, made of 6 ft. larch stakes 15 ft. apart, and wire netting 19 in. gauge, 1¼ in. mesh and 60 in. wide. This was sunk into the ground about 9 in. deep and turned inwards a further 6 in. to prevent the rabbits burrowing underneath it.

Rabbit[1] "smeuses" or outlets were set in this fence (Pl. 22, fig. 2), and consisted of a short metal tunnel, rectangular in section, set in a hole in the netting fence to provide a way in and out for the rabbits. On such a small area as ½ acre the inhabitants could not get enough food without leaving the enclosure; in fact, the enclosure was set as near to the burrows as possible to ensure that the rabbits should leave it to feed. Seven smeuses were set at regular intervals round the fence. Inside

[1] Supplied by Messrs Hydes and Wigfull, Sheffield.

each is a door pivoted at the top so that it hangs down like a flap and can easily be pushed up from either side. At first these doors were pinned up so that a permanent clear way was left through the smeuses (Pl. 22, fig. 3). After 3 weeks, when the rabbits were conditioned to using these entrances and exits from the warren, the pins were taken out and the doors left to hang (Pl. 22, fig. 4). In a short time after this the rabbits had accustomed themselves to pushing their way through the doors.

During the afternoon of the day on which it was desired to trap, a tunnel trap was fixed to each of the smeuses. This consisted of a wooden tunnel about $15 \times 8 \times 6$ in., placed outside the fence. The outside end of the smeuse was fixed just inside the tunnel with a pin passing right through both of them, and at the other end of the tunnel a sheet of plate glass was slid into grooves formed of sheet metal, so as to prevent any possible escape, while leaving the way out apparently open. Finally, the door of the smeuse was made "one-way" by a pin pushed through the top of the smeuse, down behind the door, and through a hole in the bottom (Pl. 22, fig. 5). Thus the door could open outwards, i.e. into the tunnel trap, but not inwards towards the warren. Rabbits coming out to feed at dusk nosed their way through the smeuse and were thus caught and kept in shelter until the traps were visited after dark. In this way as many as five rabbits were caught in one trap (Pl. 22, fig. 6). The glass of each trap was cleaned every time the traps were set, and, if the weather showed signs of frost, each one was smeared with glycerine, to prevent moisture condensing upon it. Trapping was usually carried out so as to catch the rabbits when they came out to feed in the evening. Traps were set in the afternoon or late evening and were visited about 2 hr. after sunset, when feeding activity had generally finished. Occasionally, good catches were obtained by setting the traps at night and visiting them in the early morning, but this was not so satisfactory.

(ii) *Marking*

The marking of wild animals falls into two classes. They can be marked with a numbered ring or disc, so that individuals can be identified again, when retrapping is carried out and the animal can be examined in the hand. Secondly, a method is now used with birds of fixing some visible mark, e.g. leg rings of different colours, so that individuals can be identified at a distance in the field. This method has many advantages, e.g. a great number of counts can be made without any disturbance of the population. It seemed advisable, therefore, to apply some such method to rabbits. Previous workers on mammals had only used such methods as leg rings (Kalabukhov & Raevskii, 1935), numbers tattooed on the ears (Allen, 1938*a*, *b*), or small aluminium ear tags (Anon, 1935). The method finally adopted, which has worked satisfactorily for 9 months, was to pin inside the ears celluloid disks about ¾ in. in diameter with serial numbers painted upon them (Pl. 22, fig. 7). Thus a feeding population of rabbits could be examined with a telescope and the numbers checked. This clearly opens up many possibilities in estimating the survival rate, in calculating total population from the proportion of marked to unmarked noted in the feeding sample, in working out relations between identified individuals, movements, feeding habits and so on. The pins used, which were analogous to paper clips only stronger, were cut out of sheet nickel, since this metal does not corrode. Figures were marked in black upon a white ground for the first hundred, and in black upon a yellow ground for the second hundred: further colours can be used as wanted.

In marking, an assistant was present to hold a torch and help. The rabbit was taken out of the tunnel trap held by the loose skin of the back with one hand, while the other hand grasped the hind legs and extended them out straight behind thus preventing any kicking. With young animals this did not matter so much, but old bucks needed a good deal of care in handling, and, if allowed to kick, could inflict nasty wounds. Adults had the head covered with a felt mask to keep them quiet. Bucks were marked on the left ear, does on the right, so that in quick field identifications the sex might be distinguished if not the number. In marking the animal a torch was held in front of the ear, so that the course of the blood vessels could be seen, and the pin pushed through from behind. Then the disk could be clipped on to the pin from the front and the two prongs of the pin splayed out to keep the disk firmly in position. Disks of ¾ in. diameter[1] were large enough for purposes of identification.

After a month or so, occasional disks began to be picked up, which had obviously been scratched or pulled out, the head of the pin having come clean through the ear. The total number of such disks found and the number of rabbits seen with holes in their ears, but no tags, could not have exceeded ten, so that the error introduced from this source into the figures must have been very

[1] The Celluloid Printers, Surbiton, supply these disks ready stamped with numbers.

small. Nevertheless, small celluloid washers ($\frac{1}{2}$ in. diam.) were at once made and fitted on to the pins so that they would lie on the outside of the ear: this effectively prevented any repetition of the previous accidents. These, however, should not be made to fit too tight, or the blood supply to the ear will be affected. The disk numbers were painted on with quick-drying cellulose paint and dried on a hot plate. They are then impermeable to weather or scratching, and the only trouble experienced in reading the disks was due to the rabbits getting them muddy. All disks were inspected and wiped, if necessary, during retrapping, while others became clean during wet weather.

Observation was carried out with a pancratic telescope ($2\frac{1}{4}$ in. object glass) from an observation post fixed up in a belt of trees about 80–100 yd. distant from the warren. A seat was fixed in position and a screen of sacks pinned up 20 ft. or so from the ground, and the observer could enter without disturbing the rabbits, by approaching behind a belt of trees.

The habitat

The work was carried out at Sheepstead Park, Abingdon, Berkshire, an area totalling about 200 acres, including, as well as the park, farmland and rough pasture, now almost exclusively occupied by rabbits. It lies just over 200 ft. above sea-level and is situated on calcareous grit, the soil being light and sandy. The writer is indebted to Mr Philip Morland, the owner of the estate, not only for his kind permission to work there, but for much helpful co-operation and discussion of general biological points concerning the rabbit.

Text-fig. 1. Plan of Sheepstead Estate with warren areas shown in black. *A*, experimental warren; *B*, nearest warren to *A*; *C*, tree-belt warren.

Text-fig. 1 shows roughly the degree of rabbit infestation of this stretch of land. The experimental enclosure is marked in field 10, and the park itself is not so heavily infested as the ground lying farther to the west. Almost certainly the warrens here are of later origin, as may also be seen from the nature of the vegetation. In fields 1–5 and 7–9 the grass is browsed uniformly short and the vegetation is characterized by such rabbit-resisting genera as *Cirsium* and *Senecio*. In fields 10 and 11 the grass is much longer and the vegetation is characterized by *Ranunculus* (chiefly *bulbosus* and *acris*). Most of the area concerned is fairly level, but the ground slopes downwards towards the south and towards

the south-east. Thus in field 1 there is a marshy patch with reeds, which provides a lying-out place in the summer, but is very wet in winter; field 10 shows a gradient in *Ranunculus* density, since they thin out towards the south side of it, where the ground is damper.

Most of the dividing lines between the fields consist of hedgerows of hawthorn (*Crataegus oxyacanthus*) and these form cover for the rabbits, being in most cases occupied by warrens (see Text-fig. 1). The big infestation between field 7 and those to the west of it is due to a thin belt of woodland, mostly consisting of elms (*Ulmus campestris*) with a certain amount of elder (*Sambucus nigra*) and elm scrub. Field 1 shows a good instance where almost the whole of the circumference is occupied by rabbit warren and colonization has taken place from these regions out into the open. These hedges are wide and untrimmed, so that they form a wide belt of cover into which rabbits can retreat. The only other cover of major importance is the summer crop of ragwort (*Senecio Jacobaea*) which grows in fields 1 and 8. This is generally cut about August, but may form a protection for young rabbits. In field 10 and also in Marcham Park immediately to the south, the warrens are overgrown with a thick cover of nettles (*Urtica dioica*), which gives cover and binds the sandy soil together, thus preventing the burrows from caving in. The north-east part of the area shown in the figure is that occupied by the farm. The further part of field 6 is arable ground without doubt heavily invaded by rabbits, while fields 7 and 9 are often used for grazing cattle. Field 1 is occasionally used for this purpose. In field 4 and to a less extent in field 3 some of the warrens have rough bushes of gorse (*Ulex europaeus*), but these have been badly eaten by the rabbits. One of the effects of rabbit infestation on this heathy part has been the reduction of the area of gorse.

Rabbits are kept down as follows. At the north end in field 5 the shooting of them is let. On the farm ferreting and wiring is carried out, and gas resorted to on occasions. The rest of the area is wired, ferreted and trapped throughout the winter. The job is rather laborious in this soil, because the burrows go to a depth of 6 ft. or more and are often distributed among strata of sandstone. A line ferret is generally used and the burrows are dug out.

Trapping at the experimental warren was started in March, and sight observations in the following month. Young were appearing during the earlier month, and they continued to be marked until the beginning of June, when breeding ceased. The number of adults in the warren at the beginning of the breeding season was estimated to be about seventy and the number of young born about 280. The greater part of these stayed within field 10, but some wandered into the wood cover between fields 9 and 10, into other warrens and into new buries. No migration was traced across the eastern boundary and practically none southward. Trapping was carried out on the average twice a week during April, May and June, once a week during July and August, and once a fortnight during September. This was done by the writer with the valuable assistance of a number of people, except during the period 18 June–9 July, when trapping was carried out by Charles Elton and R. M. Ranson. Sight observations are lacking for this period, but, apart from that gap, were carried out on an average three or four times a week.

The biology of the rabbit

(i) *Feeding areas*

These varied little during the period concerned except in so far as newly formed buries acted as new focal points. Round the experimental enclosure an area of nearly an acre acted as the main feeding ground and was sharply marked off from the rest of the field on account of the heavily grazed appearance of the vegetation. Most normal feeding took place here, but during bad weather or when the rabbits had only just come out, feeding took place round the burrows. During good weather and when the feeding urge was strong, they would go beyond this grazed area and spread out even to the edges of the field.

The warren, marked *B* in Text-fig. 1, had a feeding area southwards and westwards and so met with that of the experimental enclosure. Similarly, the area belonging to the tree-belt warren (*C*) also extended into field 10 and met those of both the other warrens. It is no doubt in this way that young rabbits are encouraged to wander from their own warrens occasionally and take up new quarters. Probably their presence in a strange warren is not so violently resented as that of old rabbits would be. Some warrens have alternative

feeding areas. This was noticeable at the tree belt warren (*C*), where the inhabitants would feed predominantly in field 9 or field 10 according to the direction of the wind. Thus, in working out the relation of the size of the feeding area to that of the warren, it is only possible to take the heavily grazed area as a standard feeding area, and add a rather arbitrary amount according to observation. From observation only about 2 acres around the experimental enclosure were regularly used by its inhabitants. So a warren of about $\frac{1}{2}$ acre with a population maintaining itself during the spring and early summer at about 150 used a total feeding area of about 2 acres. Of this about an acre was heavily grazed, the rest was used for more occasional excursions.

In other more heavily infested areas of the estate the feeding areas coalesced, and so it was practically impossible to make any estimate of the relation of feeding area to warren area. In later summer there is little doubt that feeding areas are extended merely by the greater availability of cover for lying out.

(ii) *Food and feeding habits*

In the warren itself, which was mostly covered with nettles (*Urtica dioica*) and ground ivy (*Nepeta hederacea*), both rabbit-resisting species, the main plant eaten was burdock (*Arctium lappa*), apart from scattered grasses. The only other proved food plant was buttercup (*Ranunculus bulbosus*), a leaf of which was found in the mouth of a shot rabbit. During the winter 1938–9 two snowstorms occurred, the first preceded by a long spell of frost, and during this time trees and saplings were attacked in many places. Hedgerow elm (*Ulmus sativa*) saplings seemed to be especially singled out for attack, and in some places 20–30 yd. of them had been barked. Apart from this, any timber lying on the ground, provided that it had been recently felled, was barked, and Scots pine (*Pinus sylvestris*) was invariably eaten.

Another interesting aspect of the rabbit's food and feeding habits concerns the digging of small scrapes. These are found at all times of the year, but particularly in winter: in grassland any bare patches of ground, especially fairly old molehills, are dug into, and on the edge of ploughland sometimes hundreds of these scrapes can be seen. They are characterized by their shallowness, being only 3–4 in. deep. The writer examined a great number of them in all situations and came to the conclusion that they were definitely formed for obtaining roots. In many of them on the grassland fine grass roots could be seen. It has been known for some time that hares and rabbits eat their own faeces, and Madsen (1939) has shown this to be a normal physiological process in the rabbit. It has been observed by the writer on four occasions (Southern, 1940). The importance of this process of refection may bear on the rabbit's ability to lie up for a long period, especially if prevented from feeding by bad weather or disturbed by trapping operations. One or two rabbits were trapped on a neighbouring estate under conditions which made it certain that they had been in the burrows for about 3 days. Three of these were examined and the stomach contents consisted of a mass of darkish material, brown in colour, which was almost certainly refected faeces.

Feeding may be divided into three types. (1) Casual feeding, in which the rabbit interrupts its basking to nibble idly at various plants and grasses. Burdock is eaten in this way and long grass stems; the rabbit sits down quietly to absorb the whole length of the latter bit by bit. This type of feeding occurs mostly near the entrance to the burrow,

(2) Normal feeding. This is usually true cropping and takes place mostly on the hard-grazed area round the warren. The rabbit moves slowly, stopping at frequent intervals to raise its head alertly and look round, chewing the while. An area of a semicircle is cropped by the animal turning its head, sometimes round almost to its flank; in this way a fresh movement is made in a new direction, that in which the head happens to be turned, so that a zigzag path is described. (3) Voracious feeding. This often occurs before a storm. The head is kept down the whole time and side-to-side movement is not so common, or is merely a sideways snatch. Selection of herbage, which makes for such movement in normal feeding, is in abeyance to the urge to feed quickly and the animal moves in a fairly straight line.

(iii) *Excreta*

Hendrickson (1936) was able to use faecal pellet counts as indicators of total population in the case of the cottontail. In the case of the rabbit, however, this is of no use, since the population is distributed so unevenly and lavatories are used. Generally these special patches or lavatories are to be found at a little distance from the burrow. They are often situated on old molehills, but most of them are simply patches in the longer grass, generally close to a run, but with no obvious character to suggest why they have been selected. They are easily identified by the collection of pellets and by the brown and withered appearance of the grass which extends for an area about 1 ft. in diameter. Often a single lavatory is referable to a particular burrow, but many of them give no indication of whether any special section of the population uses them.

(iv) *Feeding rhythms*

These are controlled by a great many factors, though a basic dusk and dawn feeding activity can be discerned. The various possible factors may be treated separately.

(1) *Rain.* This, contrary to many expressed beliefs, does not seem to deter rabbits from normal feeding: during observations rabbits have often been watched feeding vigorously, when the ground was very wet, and even when it was raining fairly hard, although heavy rain will drive them into the cover of the burrows. Feeding at dawn usually occurs long before the dew has dried from the grass, and at this time they have been observed feeding among the long grass at considerable distances from the warrens. Possibly they prefer to feed on young grass stems at this time, which offer less surface for condensation.

(2) *Temperature.* This seems to affect feeding little unless cold is combined with wind. Rabbits have been seen out in great numbers just after dusk, when a heavy frost was on the ground.

(3) *Wind.* Any strong wind decreases feeding activity, and most notably a cold east wind: at these times rabbits are particularly susceptible to "scares".

(4) *Barometric pressure.* All the occasions when rabbits have been noted feeding voraciously have been prior to thunder or rainstorms, so it is possible that they are sensitive to changes in barometric pressure. On one day when a heavy thundercloud blew up, practically all the rabbits came out and fed furiously until they were driven in by the breaking of the storm.

(5) *Snow and other bad conditions.* Vigorous feeding is often observed after a spell of unfavourable weather, e.g. when snow has been on the ground for several days, or if an unusual amount of continuous rain has fallen.

(6) *Sunshine*. Feeding activity is not affected by sunshine. On misty August mornings activity is always at a maximum just after dawn, and by the time the sun has broken through, most of the rabbits above ground are basking and washing themselves. On the other hand, sunshine is obviously welcome for the last two processes, and rabbits will lie about on the warm bare earth outside the burrows with their underparts exposed to the warmth just like a dog on a hot pavement.

Records are being maintained of feeding activity to see whether positive correlation can be established with any of the factors considered above, but it is difficult to collect a large body of facts, except over a considerable period, on account of disturbance due to trespass and trapping operations.

(v) *Sex differences*

Among morphological differences the shape of the head is sometimes useful in the field. Most old bucks can readily be recognized by the bluntness and width of the muzzle, though the diagnosis becomes less easy in the case of young bucks, which have just as pointed muzzles as the does. Behaviour is not a certain criterion either, but as a general rule it may be said that does, when coming out to feed, go straight away from the warren to the feeding grounds, while the bucks spend more time feeding just around the burrows (cf. Niethammer, 1937). Diagnosis of the sex of small young may be mentioned, since there seems to be a slight divergence of opinion in published accounts (Sawin *et al.* 1938; Hammond, 1936). Eversion of the urinogenital papilla in the smallest rabbits caught (0·15 kg.) will always show a rounded aperture in the males as opposed to a slit-like one in the females. Care should be taken also with oestrous does, since the clitoris is almost as eversible as the penis, and may be mistaken for one if examination is superficial.

(vi) *Age determination*

This is a difficult problem, especially to the worker in the field. Generally, rabbits of the year may be distinguished from adults, and even through into the next spring. The ears of young rabbits are not so leathery and tough and are generally colder than those of old ones. The nails also are shorter and less ragged. Determination of the age of old rabbits is very difficult. In the course of weighing some hundreds of them, dead as well as alive, it has been noticed that the weights tend to fall into groups. This may give some clue to age, but more data are needed to give statistically significant results.

Figures relating to 128 young rabbits (62 males and 66 females) whose weight did not exceed 0·95 kg., are of interest. The weight was taken to the nearest 0·05 kg. and trapping of the particular rabbits which gave these figures lasted from 30 March until 25 July. The frequency of retrapping was:

Times caught	Males	Females	Total
1	39	44	83
2	11	16	27
3	7	3	10
4	2	2	4
5	3	1	4
Total	62	66	128

These rabbits were of various ages when first trapped, but were all young. The mean weight of the 62 males when first caught was $0{\cdot}368 \pm 0{\cdot}015$ and of the 66 females $0{\cdot}381 \pm 0{\cdot}0199$ kg. The smallest weight at which an animal was commonly caught was 0·2 kg., which is

probably equivalent to the time of emergence 3 weeks after birth. Forty-five of these rabbits were trapped more than once, and when the weights of those caught four or five times were plotted against time, taking in each case $t=0$ at the date of the first trapping, it was found that the points for each individual lay nearly on a straight line and that the slopes of the individual lines were roughly parallel. Thus within the range 0·2–0·95 kg. the regression of weight on time, and therefore age, could be taken as being roughly linear. This is confirmed from data for laboratory stocks with a similar adult weight to that of the wild rabbit (Castle, 1929, 1931; Kopeć, 1927; Pease, 1928; Wilson, 1930; Dunlop & Hammond, 1937).

If this be so, and if environmental conditions did not alter throughout the series of trappings, it is possible to determine the daily rate of increase. One doe out of the twenty-two was eliminated, because she was not recaptured until 138 days after the initial trapping. The day on which an individual was first trapped was called $t=0$. The time between this date and the recapture dates was then grouped into 10 day intervals with the following results:

Recaptured between days	No. of rabbits	Mean weight $t=0$ g.	Mean weight at recapture g.	Mean time of recapture days	Daily rate of increase
1–10	25	0·350	0·394	5·3	0·0083
11–20	9	0·361	0·500	14·7	0·0095
21–30	6	0·283	0·541	25·8	0·0100
31–40	9	0·350	0·722	34·8	0·0107
41–50	11	0·336	0·802	47·0	0·0099
51–60	2	0·350	0·850	53·5	0·0093
					Mean = 0·00961

Calculations were made comparing the mean daily rate of increase in males and females, and though a slight difference was found this was not statistically significant. If straight regression lines be fitted by the Method of Least Squares to the data tabulated by Castle (1929) for his race of small males and females between the weights of 0·3 and 0·9 kg., which are equivalent to the ages between 30 and 100 days, the equations are:

$$\text{Males:}\quad w=0{\cdot}03694+0{\cdot}008438X,$$
$$\text{Females:}\quad w=0{\cdot}07547+0{\cdot}008424X,$$

where w is the weight in kilograms and X is the age in days. For a particular age the males are on the average somewhat smaller than the females, as indicated by the difference between the first constants in the above equations, while the daily increase is to all intents and purposes the same for both sexes and of much the same order of magnitude as that we have estimated for these field data.

The smallest weights recorded at the first trapping were one female of 0·15 and two males of 0·2 kg. These individuals must have been taken very shortly after emergence from the nest, or, so far as our information goes, some 3 weeks or so after birth. If we assume that a weight of 0·2 kg. is equivalent to the age of 3 weeks, which agrees closely with the laboratory data published by Castle (1929, 1931), Kopeć (1927), Pease (1928), Wilson (1930), and Dunlop & Hammond (1937), the equation defining weight in terms of age, still keeping within the limits of 0·2 and 0·95 kg., as

$$w=0{\cdot}200+0{\cdot}00961\,(X-21)$$

or

$$X=0{\cdot}188+104{\cdot}06w,$$

where w is the weight in kilograms and X is the age in days. Thus, knowing the weight of an animal on a certain date, it should be possible to estimate its age and thus the date of its birth.

There may, however, be large errors in this type of estimation. Briefly, the error of the age estimated from the weight will depend both on the variability of the individual weights around the mean weight for age given by the regression line and also upon the value of the mean daily rate of increase. If for a given age we have a distribution of weights whose standard deviation is S_w, the standard deviation of the age distribution estimated from the weights will be S_w/b, where b is the mean daily rate of increase. In addition, error will be introduced according to the deviation of the mean of the weight distribution from the regression line. In the case of animals caught at different seasons of the year, the distribution of ages so estimated will overlap the true unknown distribution of ages and their time of birth. Thus Table 1 can only serve to indicate the outside limits of time during which the young were born. A third source of error is the assumption that a weight of 0·2 kg. is equivalent to an age of 3 weeks. This may be above or below the mean weight of the population at this age; the effect of this deviation is to shift the distribution of ages either to the right or left along the time axis.

TABLE 1. *Calculated distribution of births in 3-week periods of young marked rabbits*

Date	Males	Females	Total
6 Feb.–26 Feb.	8	8	16
27 Feb.–20 Mar.	16	14	30
21 Mar.–11 Apr.	14	12	26
12 Apr.– 2 May	9	16	25
3 May–23 May	13	13	26
24 May–13 June	2	3	5
Total	62	66	128

(vii) *Reproductive physiology*

The litter rate. Two things are probable: first, that the size of litters increases as the breeding season progresses, and, secondly, that environment may control the litter rate.

As regards the first point, most evidence is hearsay, but the writer collected a series of embryos in the spring of 1939, and from thirty-four pregnant does obtained between 12 Feb. and 2 Mar. an average litter number of 3·94 was found. From the figures taken from the experimental warren (see later) 280 young were born in the season from about thirty-six does, exclusive of mortality before weaning. This implies an average of about eight young weaned to each doe: these are probably distributed over two litters in most cases in the proportion of 3 to 5 for the first and second respectively, if we may take into consideration the early litter rate from the thirty-four does mentioned above.

As to the influence of the environment, Lockley (1939) mentions that on Skokholm Island off the coast of Pembrokeshire the litter rate averages about four throughout the year. The rabbits are small in size and suffer through lack of food, especially in the winter. Reproductive activity is also checked in other ways: breeding does not begin until April, and the first young do not come into breeding condition until the following year.

Hammond (1934) has shown that infertility in the rabbit may be genotypic, and is effected mainly in two ways: the number of ova shed may be low or survival during pregnancy

may be low. In this case in populations where nutrition is bad there may even be selection for a low litter rate. On the other hand, the factor for foetal atrophy at least is recessive, so its appearance may simply be due to isolation of the population and to inbreeding. This explanation would probably apply to the case of the Skokholm rabbits, but with continuously distributed populations it becomes important to know what degree of isolation exists between them. Thus, the figures given on p. 519 showing a low diffusion rate of rabbits from the experimental warren are interesting.

One of the points most needing elucidation is the control of oestrus in the does. This is no doubt mediated by the level of follicle-stimulating hormone in the anterior pituitary, but the actual control would seem to be complex. Clearly adverse weather conditions can have an effect: at the experimental warren breeding ceased entirely in June. Barrett-Hamilton & Hinton (1910–21) state that does become capable of breeding in 3 months, so that the whole of the young stock of the warren should have been breeding by July or August. The gestation period is well known to be 28–30 days, but the weaning period (i.e. to time of emergence) is not so well known. Barrett-Hamilton & Hinton (1910–21) state that young rabbits can look after themselves at the end of 3 weeks, and for *Sylvilagus*, Allen (1938*b*) gives 16, "or a few more" days. Weights of the smallest caught suggest 3 weeks (see p. 516).

The total number of young rabbits marked in 1939 was 142, the sex ratio being males 47·9 %, females 52·1 %. In *Sylvilagus floridanus* the ratio is similarly weighted in favour of the females, though not to the same extent; Gerstell (1937) found that of 6394 cottontails 49·2 % were males and 50·8 % females.

(viii) *Colonization*

A population on favourable ground, whose increase is unchecked, spreads rapidly, and the general method of extending the warren area is by means of the stops that the does construct away from the main warren for the purpose of breeding. A number of these are made round the periphery of the main warren and when breeding is over the young stay there and extend the burrow with further ramifications until contact is made with the main warren. This separate and smaller focus then becomes incorporated and so the whole area grows. In addition to this, however, some does go a considerable way from the warren to make their stops, and, if these are on open ground, they gradually become turned into small warrens when breeding is over. Eventually an area which had a number of well demarcated warrens may turn into general warren, such as may be seen in fields 1 and 2 at Sheepstead (Text-fig. 1).

Often cover seems to be the determining factor in establishing a new focus. In parkland the cover afforded by the tree roots and by the nettles that quickly grow up when the ground has been disturbed seems to attract rabbits, and it may even be noticed that where the shelter cast by a tree is asymmetrical, e.g. owing to a large branch having fallen off on one side, the warren area established is similarly asymmetrical. This shows that in some cases at least the shelter afforded by the tree is the main factor determining colonization, probably by the effect on the ground beneath and the vegetation. Colonization in many cases seems to advance out onto open ground from hedgerows, the latter acting as reservoirs, even though the open warrens may be destroyed. Very possibly the common practice of

hedging, prevalent in agricultural areas of Britain, is responsible for carrying a population of rabbits, where otherwise they might find it difficult to get sufficient cover.

(ix) *Disease and parasites*

The only disease that was encountered at Sheepstead in 1939 was coccidiosis (*Eimeria* sp.). This broke out fairly heavily on the more heathy ground, i.e. fields 1, 2, 3, 7 and 8, but never reached fields 9, 10 or 11 (see Text-fig. 1). Neither was it recorded in Marcham Park to the south of Sheepstead. It appears from this that such outbreaks may be sporadic and not travel far from their place of origin. This seems to corroborate the evidence offered that a considerable degree of isolation exists between populations of wild rabbits. It should be noted that during May and early June of 1939 the weather was fine and dry, but after that wet conditions set in. This combination of conditions may be particularly favourable to spread of this disease by initiating sporulation of the oocysts. Naumov (1939) collects a great deal of evidence to show that coccidiosis among other diseases is commoner in hares when the weather has been wet.

Rabbit fleas (*Spilopsyllus cuniculi* (Dale)) were found fairly commonly on the trapped animals, but there was variation in the degree of infestation according to season and according to sex. In April and early May the only animals with anything like heavy infestations were the adult does. From about the middle of May onwards the young and then the adult bucks begin to be infested, and a peak is reached about the middle and end of July. After that the numbers decrease gradually until in September only a few odd ones are found. These fleas are mostly found on the ears of the rabbits, but a number were usually to be found about the head region. These were not apparent at first, but blowing on the fur was sufficient to dislodge them and make them come to the surface.

(x) *Aberrant types*

White spots on the forehead occurred sometimes, though not very commonly. Four of the 142 young caught were marked in this way. Among the rabbits inspected early in the season for embryo counts, two out of 100+ were found to have bright yellow fat. This factor is fairly well known in domestic rabbits. Apart from this the only mutant seen was a single sandy coloured rabbit in warren B, field 10 (see Text-fig. 1).

(xi) *Migration*

A certain amount of information was gathered by tracing marked animals, either because they were reported dead at a distance or because they were observed feeding at other warrens. All such animals traced were young of the year. Twenty-one emigrants were recorded in this way and of these four emigrated in March, two in April, two in May, three in June, two in July and five in August (one returned again). Thus a steady trickle of young rabbits was moving out all through the breeding season, though it never reached very large proportions. The importance of these movements must also depend upon the distance covered, which was in no case very great. Spread was mostly to nearby warrens, cover and fresh buries, started in the same field. The last two allow no reciprocal immigration to make good the deficit. Of the above records six rabbits had moved less than 70 yd., nine less than 140 yd., three less than 210 yd., and three less than 280 yd. Niethammer (1937) gives figures for adults which show that they are very stationary.

Vital statistics of the rabbit

(i) *Nature of records taken*

At the outset only marked rabbits will be considered. After this, results can be extrapolated to give figures for the total population. The basic data collected are the marking times of 162 rabbits trapped in the experimental enclosure, and observations showing the survival times of these rabbits. The moment when a marked rabbit dropped out of the observation records, taken two or three times a week, is taken as the time of its *disappearance*, not necessarily of its death. It is not possible to distinguish the death rate from the emigration rate, so that the two have been merged together for the purpose of this paper and are referred to as the *disappearance rate*.

The term *appearance rate* must not be confused with the marking rate, which, though related to the appearance rate, can only be so in an indeterminate way. Appearance rate may be obtained by calculating the frequency distribution of births among the marked population and this can only be done within certain limits of error (see p. 517). Though appearances are referable to the time of birth, the appearance rate is not the same as the birth rate, since only animals successfully weaned were exposed to the risk of being trapped.

Clearly some convention has to be adopted for simplifying the data. Rabbits were appearing and disappearing from the population continuously, but for present purposes it must be assumed that this occurred discontinuously. In this way a sort of balancing up of accounts can be made at stated intervals, and the changes taking place between these intervals shown with clearness. The periods adopted, dictated chiefly by the frequency of the record taking, have been three-week ones, and are allotted as follows:

Period 1	16 Jan. – 5 Feb.	Period 7	24 May–13 June
Period 2	6 Feb.–26 Feb.	Period 8	14 June– 4 July
Period 3	27 Feb.–20 Mar.	Period 9	5 July–25 July
Period 4	21 Mar.–11 Apr.	Period 10	26 July–15 Aug.
Period 5	12 Apr.– 2 May	Period 11	16 Aug.– 6 Sept.
Period 6	3 May–23 May		

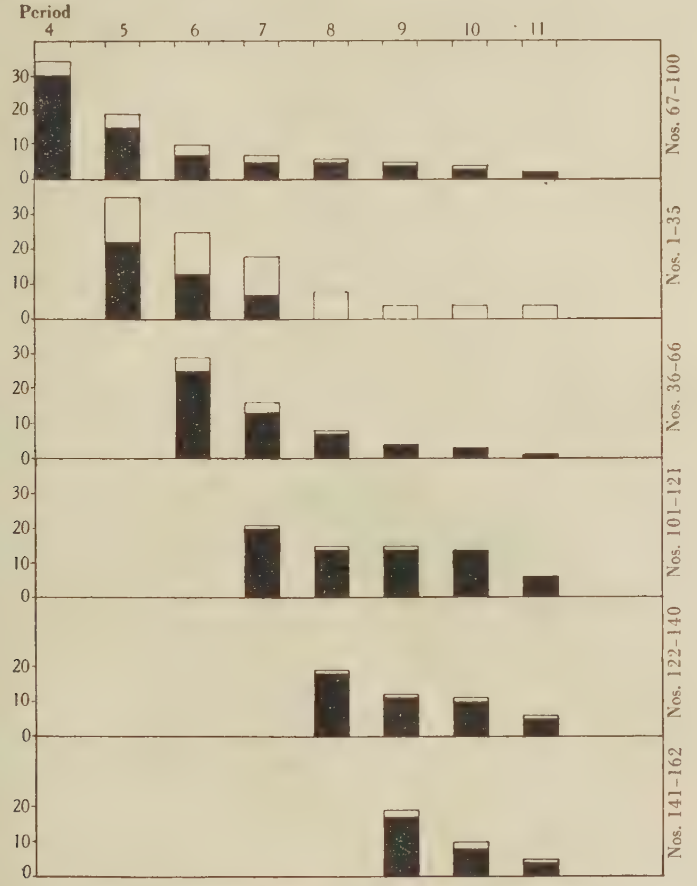
Under this convention it is assumed that the number of animals marked during any period were all marked on the middle (11th) day of it, so that they all come into consideration at the same time. Similarly, all rabbits appearing and disappearing are assumed to have done so on this one day.

(ii) *The survival rate*

So long as it is remembered that part of the rabbits that disappeared were also "survivors" in the strict sense, though not included in this survival rate, it is a matter of straightforward examination of the observation records. Each marked rabbit is followed through until it drops out of the observations, the number of periods during which it was recorded being counted as the time of survival. The number of survivors from each period (i.e. regarded as marked at the middle of that period) can thus be traced throughout the time concerned and may be shown graphically (see Text-fig. 2).

In Text-fig. 2 the adults are shown separate from the young. Since their survival rate is higher than that of the young, their inclusion in the figures without distinction would

give a false extension to the survival rate. The main thing that this figure brings out is the high disappearance rate which occurs at the beginning or soon after the beginning of the season. All the young rabbits marked in period 5 have disappeared from the population by period 8, i.e. in 9 weeks. Those marked in period 4 also show a sharp falling off in the same time. This must be attributed mostly to a heavy mortality in the stages soon after



Text-fig. 2. Survival of young and adult rabbits at 21-day intervals (21 March–6 Sept.). Young below, adults above.

weaning, since figures for emigration (see p. 519) show that there was no particularly marked outward movement at this time. This contrasts sharply with the persistence of rabbits marked at periods 7, 8 and 9, where the falling off is more gradual. Perhaps the last of these (period 9) should be excepted, but, again referring to the emigration figures, this is the period when emigration was most marked, and when animals tended to lie out on account of the fine weather and abundance of cover.

(iii) *Distribution of births and disappearances*

Table 1 shows the calculated distribution of births in a number of young marked rabbits. From the starting point it should be possible also to calculate the distribution of disappearances according to age. However, the errors involved in the double process are so many that it is impossible to give definite figures. General tendencies are all that can safely be mentioned, and these may in time be confirmed by future data.

Thus the age at which the majority of young rabbits disappear is about 64–84 days (between 3 and 4 weeks). On either side of this period the numbers fall off rapidly. If the complementary calculation be made showing the age composition of the survivors at different periods, it can be seen generally how the influx of young rabbits into the population dominates each group trapped down to period 7, when the number of births has fallen off. Even more interesting is the age composition of the survivors at the end of the season. Nine of the eighteen that remained were born about period 5, while only four were older than this. Thus fourteen out of the eighteen marked survivors were probably young born in the later litters, and by far the greater part of the earlier young had disappeared.

(iv) *Turn-over in the population*

So far only the marked population has been dealt with. These results can be extrapolated to give figures relevant to the total population. Table 2 shows the proportion of marked to total (marked and unmarked) rabbits seen during the sight observations. Periods 4, 8 and 9 have unfortunately few observations, but the general trend of the figures is clear. The rise in proportion towards the end of the season was due, not to increased trapping intensity, but to the dying away of the influx of young born. Knowing therefore the proportion which the marked animals represent in a feeding sample, and knowing the total number of animals marked, the calculation of the total population is simple. The composition of the population at each period can then be determined and the amount of change between each shown.

TABLE 2. *Observation ratio*

Period	Total counted (aggregate of separate counts)	Mean proportion of marked to total rabbits
4	14	0·28
5	379	0·33
6	214	0·39
7	323	0·37
8	56	0·44
9	76	0·52
10	338	0·55
11	205	0·56

However, adults and young are not trapped with equal ease, and therefore it is not permissible to calculate the total population of adults from the ratio $\frac{\text{marked rabbits}}{\text{total rabbits}} \times$ adults marked. The ratio marked/total rabbits refers to feeding samples, and may best be termed the "observation ratio". However, early in the season it is easy to distinguish between adults and young when they are out feeding, so the initial figure for adults can be obtained in this way. This is all that is wanted, since the number of adults is probably not subject to addition, as in the case of the young, but only to subtraction, owing to death and

emigration (the latter is probably nil). The number of adults is therefore calculated by taking the subsidiary observation ratio $\frac{\text{marked adult rabbits}}{\text{total adult rabbits}}$ and multiplying this by the total number of adults marked. The calculation of the composition of one period from that of the previous one is done as follows:

	Period A	Period A_1
Total population	T	$T_1=\dfrac{N+[M-(x+y)]}{p_1}$
Adults	t	$t_1=t-\dfrac{xt}{m}$
Young	$(T-t)$	
Marked population	M	$M_1=N+[M-(x+y)]$
Adults	m	$m_1=n+(m-x)$
Young	$(M-m)$	
Observation ratio	p	p_1

where x = the number of marked adults disappearing from the population between periods A and A_1, y = the number of marked young disappearing in the same time, and N = the number of rabbits marked between those periods (divided into n adults and $N-n$ young). The figures for period A are simply determined as mentioned above, since $M/p=T$. The figures for the marked population in the next period are simply calculated from the known values of x, y and N, and the total population for that period determined in the same way, $M_1/p_1=T_1$. The full results of these calculations are given in Table 3. It is noticeable that in spite of the large number of young born during the season the total population of the warren never rose above 162. This shows that constant change is going on the whole time.

TABLE 3. *Composition of the population from period to period*

Period ...	4	5	6	7	8	9	10	11
Observation ratio (p)	0·28	0·33	0·39	0·37	0·44	0·52	0·55	0·56
Marked adults disappearing (x)	—	0	2	4	6	5	1	2
Marked young disappearing (y)	—	15	18	20	19	11	12	20
Additional rabbits marked, adults (n)	—	13	4	1	1	2	—	—
Additional rabbits marked, young ($N-n$)	—	22	25	20	18	17	—	—
Additional rabbits marked, total (N)	—	35	29	21	19	19	—	—
Marked population, adults (m)	4	17	19	16	11	8	7	5
Marked population, young ($M-m$)	30	37	44	44	43	49	37	17
Marked population (M)	34	54	63	60	54	57	44	22
Total population, adults (t)	70	70	62	49	31	17	15	11
Total population, young ($T-t$)	51	93	99	113	92	92	65	28
Total population (T)	121	163	161	162	123	109	80	39
Total adults disappearing	—	0	8	13	18	14	2	4
Total young disappearing	—	25	45	45	49	24	27	37
Total rabbits disappearing	—	25	53	58	67	38	29	41
Disappearance rate	—	0·21	0·33	0·37	0·41	0·31	0·23	0·50
Number of young appearing	—	67	51	59	28	24	0	0
Appearance rate	—	0·55	0·31	0·37	0·17	0·19	—	—

It is unfortunate that figures were not obtained previous to period 4, since the first figure given for total population contains a large number of young. However, it is probable that not much mortality occurred among the adults that were first occupying the warren, so

that the initial population could not have been greatly above the seventy adults shown in the first column. By the end of the season, largely owing to a rapid decline in periods 9, 10 and 11, the total population stood at only forty. This gross loss is more apparent than real, since many of the rabbits are lying out at this time, and doubtless with the approach of autumn a number will come back to the warren. This makes it all the more desirable to have figures for the non-breeding season. Although the greater part of the wastage occurred among the young rabbits, it is nevertheless notable that only eleven of the original seventy adults survived. This means that the life span of the rabbit in nature is probably quite short, the majority of them living only about a year. This is rather surprising in view of the age to which they live in captivity.

The lower lines of figures in Table 3 apart from those giving the values of x, y and N, give further details of what was happening in the population. Up to the time when appearances ceased, the total number of adults and young disappearing are calculated from x and y; the total number of adults disappearing $= xt/m$, and that of the young $= [y(T-t)]/M-m$. In this way the sum total of the young disappearing in each period plus the number remaining in period 11 equal the number of young produced, i.e. 280. The appearance and disappearance rates can be calculated from these data, and show that the highest disappearance rate occurred in period 11, whereas the greatest number disappeared in period 8. Similarly, it may be shown that young continued to arrive into the population until period 8, about 6 weeks after the latest of them were born, which suggests that weaning may take longer in some cases than the 3 weeks suggested by some authorities. This of course may particularly apply to the later litters.

(v) *Mortality*

Twenty records of deaths were investigated during the season, of which the cause of death was ascertainable. Five of these rabbits were killed by complications ensuing from the trapping procedure: one of them was strangled in the netting fence, and the others were damaged by being in the same trap as a large buck, and had suffered severe enough damage to necessitate being killed, or else were found dead afterwards. It is possible that this factor may operate under normal conditions to a certain extent. Nine out of the twenty records were due to dogs and cats, and all of them were quite young (up to 2½ months old). Of the others, of which the cause of death was ascertainable, one was caught in a late-set gin-trap, another was shot by the keeper of the neighbouring Marcham Park and four were almost certainly killed by foxes. There is no doubt that the most important factor in mortality at this warren is stray dogs and cats.

(vi) *General conclusions from the figures*

The total estimated productivity of thirty-six does in the experimental warren in 1939 (possibly not a normal year) was 280 young weaned, or just under eight each, born from February to June. Of these 252 disappeared during the season, some of which may subsequently return when the winter drives them back to the warren, and others of which have taken up permanent quarters elsewhere. The main loss was concentrated in May and June and fell chiefly upon young rabbits about 3–4 weeks old. Of seventy adults fifty-nine disappeared, probably all of them being killed, since none of them was traced to a distance. This means that a great deal of loss in the breeding stock has to be made good every year,

and it is difficult to see how the stock manages to survive the mortality of the winter. As mentioned before, however, numbers of young rabbits must be spread over the neighbourhood in late summer, to return later, although this still means that the majority of the next year's breeding stock will consist of this year's young.

Summary

1. A new technique is described for trapping wild rabbits alive and marking them with visible identification tags, which can be examined from a distance with a telescope.

2. A general description of the habitat is given.

3. The general biology of the wild rabbit is dealt with. Food, feeding habits and ranges are discussed; refection is found to be common in wild rabbits. The age-weight relationship in young rabbits is shown to be roughly linear, giving a method of calculating age from weight. In reproductive physiology, some data on litter rates are considered, the sex ratio is shown to be slightly in favour of females. Figures from recoveries of marked animals are given showing the amount of migration that takes place.

4. Vital statistics taken from sample counts made with a telescope show the rate of survival from March to September, the period with which this paper is concerned, the distribution of births and disappearances, and by extrapolation of data for marked rabbits to the whole population give details of the turnover in individuals (i.e. the composition of the population at successive points in the season).

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REFERENCES

Allen, D. L. (1938*a*). Vertebrate fauna of a 500-acre farm. *Ecol. Monogr.* **8**, 347.

—— (1938*b*). Breeding of the cottontail rabbit in southern Michigan. *Amer. Midl. Nat.* **20**, 464.

Anon. (1935). [Experiment in marking the Arctic fox (*Alopex lagopus*) on Novaya Zemlya.] *Bull. arctic Inst., Leningr.*, **9**, 281. (In Russian; summary in English.)

Barrett-Hamilton, G. E. H. & Hinton, M. A. C. (1910–21). *A History of British Mammals*, **2**, 176. London.

Castle, W. E. (1929). A further study in size inheritance in rabbits with special reference to the existence of genes for size characters. *J. exp. Zool.* **53**, 421.

—— (1931). Size inheritance in rabbits: the backcross to the large parent race. *J. exp. Zool.* **60**, 325.

Dunlop, G. & Hammond, J. (1937). The growth and proportions of the rabbit's ear in relation to body weight. *J. Genet.* **34**, 463.

Gerstell, R. (1937). Management of the cottontail rabbit in Pennsylvania. *Penn. Game News*, **7**, 7.

Hammond, J. (1934). The inheritance of fertility in the rabbit. *Harper Adams Util. Poult. J.* **19**; Poultry and Rabbit Conf. No. Aug. 1934.

—— (1936). Pregnancy in the rabbit. *Kongressbericht*, B. 1, 6. Weltgeflügelkongress, Berlin and Leipzig.

Hendrikson, G. O. (1936). Summer studies on the cottontail rabbit (*Sylvilagus floridanus mearnsi* Allen). *Iowa State Coll. J. Sci.* **10**, 367.

Kalabukhov, N. & Raevskii, V. (1935). [The study of migrations of ground squirrels (*Citellus pygmaeus* Pall.) in the steppe area of Northern Caucasus by the banding method.] *Problems of Ecol. and Biocenol.* **2**, 170. (In Russian; summary in English.)

Kopeć, S. (1927). The morphogenetic value of the weight of rabbits at birth. *J. Genet.* **17**, 187.

Lockley, R. M. (1939). My island and our life there: 19, an experiment in the extermination of rabbits. *Countryman*, **19**, 557.

Madsen, H. (1939). Does the rabbit chew the cud? *Nature, Lond.*, **143**, 981.

Naumov, S. P. (1939). [Fluctuations in numbers among hares.] *Problems of Ecol. and Biocenol.* **5–6**, 40. (In Russian; summary in English.)

Niethammer, H. (1937). Ergebnisse von Markierungsversuche an Wildkaninchen. *Z. Morph. Oekol. Tiere*, **33**, 297.

Pease, M. S. (1928). Experiments on the inheritance of weight in rabbits. *J. Genet.* **20**, 261.

Sawin, P. B., Green, E. L., Johnson, R. B. and Wightman, J. C. (1938). Sexing newborn rabbits. *J. Mammal.* **19**, 109.

Southern, H. N. (1940). Coprophagy in the wild rabbit. *Nature, Lond.*, **145**, 262.

Wilson, W. K. (1930). Some husbandry conditions affecting growth in the rabbit. *Rep. Proc. 4th World's Poultry Congr.* p. 949.

EXPLANATION OF PLATE 22

Fig. 1. General view of rabbit warren.

Figs. 2, 3. Smeuse let into the netting enclosure. Door pinned up at first to give open entrance and exit.

Fig. 4. Smeuse with door hanging.

Fig. 5. Smeuse made one-way by a pin pushed through the top, down behind the door, and through a hole in the bottom.

Fig. 6. Rabbit being removed from box trap.

Fig. 7. Rabbit marked by pinning a numbered celluloid disk inside ear: holding to prevent kicking.

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Fig. 1

Fig. 4

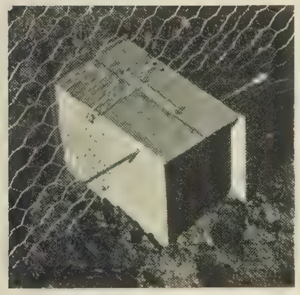

Fig. 2

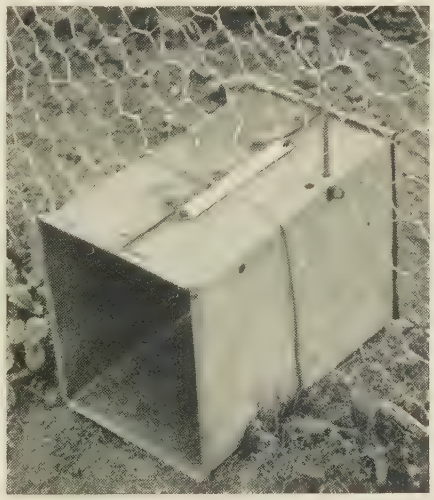

Fig. 5

Fig. 3

Fig. 7

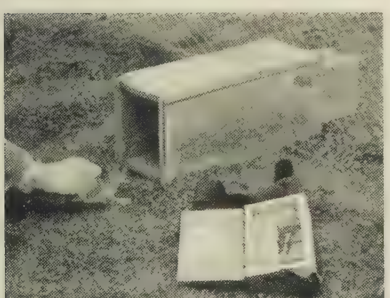

Fig. 6

SOUTHERN.—The Ecology and Population Dynamics of the Wild Rabbit (pp. 509–526)

SHEEP BLOW-FLY INVESTIGATIONS

VIII. OBSERVATIONS ON LARVICIDES AND REPELLENTS FOR PROTECTING SHEEP FROM ATTACK

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Sheep only become infested with maggots when certain conditions are present which render them susceptible to attack, viz. the presence of bacterial action and moisture in the fleece (Davies & Hobson, 1935); these may arise in various ways. The incidence of attack in the field is influenced by climatic conditions and other factors. Consequently, the testing of substances in the field is difficult and it was necessary to devise artificial methods for determining the value of protective chemicals. The discovery that certain substances attract blowflies to oviposit on sheep supplied a method of making sheep artificially attractive (Hobson, 1935, 1936). However, maggot infestation does not invariably follow as the conditions in the fleece may be too dry, but MacLeod (1937) has described a convenient method of producing artificial infestation, which consists essentially of placing eggs or young larvae on the skin of the sheep under a pad of moist cotton-wool. MacLeod (1938) has also examined certain carbolic and arsenic dips by these methods and found them suitable for evaluating the repellent and larvicidal properties of dips; his paper includes an admirable discussion on the general question of protecting sheep against blow-flies and this subject will not be elaborated here. Broadly speaking, a fly dip may protect sheep in three ways: (1) by preventing the development of susceptible conditions; (2) by repelling the flies and thus preventing the laying of eggs in the fleece; (3) by killing the eggs or young maggots in the fleece. In the present work, the main lines of investigation have been the last two, by means of repellent and insecticidal action.

Experimental

Larvicides

Sheep were dipped in the experimental dips and tested at intervals by inserting eggs on the skin under moist conditions; wool samples were also collected from the base of the fleece for laboratory tests. In the case of organic compounds, the repellent properties were tested at intervals by placing an attractive solution of indole and ammonium carbonate in the fleece (see p. 529). The method of producing artificial myiasis was the same as that described by MacLeod (1938) except that eggs were used in preference to first instar larvae. A cluster of about 150 *Lucilia sericata* eggs was taken for each experiment, the eggs being kept overnight and placed on the sheep next day. At the selected spot on the back of the sheep, the skin was moistened and gently rubbed; the egg batch was then placed on the skin and covered with a plug of damp cotton-wool which was kept in place by tying the surrounding wool with string. After 2 days the plug was removed to determine whether the larvae had produced a wound. Control experiments were carried out with untreated sheep; this method proved very reliable and myiasis was successfully produced in about 90% of the control tests. In addition to the myiasis experiments, the larvicidal properties of the wool were tested in the laboratory by moistening the wool with an equal weight of serum and feeding young larvae on the mixture for 6 hr. at 37° C. This method proved useful for determining the persistence of the poison in the fleece.

Three sheep were used for each experimental dip, the animals being immersed for 1 min. The organic compounds were used in the form of emulsions and in most cases refined petroleum oil was included. Insoluble substances were pasted with water and a proprietary wetting agent, Agral 2, was added to the dip at the rate of ¼ lb./100 gal.

Results

The results are collected in Table 1. The insoluble arsenites of lead and calcium had about the same effect as the proprietary arsenic-sulphur dip which contains a large proportion of water-soluble arsenic; the significance of this will be discussed later. Sodium silicofluoride proved ineffective despite the fact that it had been shown to be toxic to

Table 1

Dip	Strength	Duration of repellent action weeks	Protection against experimental myiasis weeks	Wool toxicity
Proprietary arsenic-sulphur powder	0·2 % As_2O_3	—	3–4	3
Lead arsenate	,,	—	2	1
Lead arsenite	,,	—	3–4	3
Calcium arsenite	,,	—	3–4	3
Sodium silicofluoride	1 %	—	< 1	0
Barium silicofluoride	1 %	—	2–3	2
Sulphur dip (proprietary)	—	< 1	< 1	0
Derris extract (proprietary)	—	< 1	2	1
Aberdeen University dip	—	< 1	1	—
n-Butyl carbitol rhodanate	0·5 %	1½	1–2	D
Dodecyl rhodanate	0·5 %	6	6–7	3
Dodecyl rhodanate	0·2 %	1½	1–2	1
Benzyl cyanide	0·2 %	1½	1–2	D
Benzthiazyl (2) methyl sulphide	0·2 %	1	1–2	D
Phenyl isothiocyanate	0·2 %	1	1–2	D

Wool toxicity

3 = wool highly toxic 6 weeks after dipping.
0 = wool had little or no toxicity 1 week after dipping.
1, 2 = toxicity intermediate between 0 and 3.
D = wool highly toxic after dipping, innocuous 1–2 weeks later.

L. sericata larvae in laboratory trials. Barium silicofluoride gave a better result, but, as a very high concentration was used, this material does not appear to be a likely substitute for arsenic. Excellent results were obtained in the first test with dodecyl rhodanate (lauryl thiocyanate) at 0·5 %, but at 0·2 % this substance afforded poor protection. Severe skin injury was observed in the sheep dipped in 0·5 % dodecyl rhodanate several weeks after dipping; as the skin was affected only along the back and not on the flanks, it seems probable that the emulsion broke during dipping and that a large amount of oil collected on the back where the tests were carried out. Normal butyl rhodanate (β-butoxy-β'-thiocyano-diethyl ether) was tested in the form of "Lethane 384", a proprietary substance containing 50 % of the poison mixed with refined petroleum oil; the results of the wool tests show that this substance was not stable in the fleece. The other synthetic insecticides tested were supplied by Imperial Chemical Industries, Ltd. In laboratory tests these compounds proved highly toxic to blow-fly larvae; however, in field tests with various types of emulsions, the period of protection was short, apparently owing to the poisons disappearing from the fleece. With regard to the Aberdeen University fly dip, suggested by Moore (1937), the

results obtained confirm the conclusions of MacLeod (1938) that this dip has poor larvicidal and repellent properties and is effective in virtue of its antiseptic properties.

Repellents

Preliminary results have already been described with repellent substances mixed with the attractant (Hobson, 1937). In the present experiments, sheep were sprayed or dipped with the repellent and tested at intervals by inserting in the fleece a small piece of cotton-wool soaked with the attractive solution. This solution contained 2% ammonium carbonate and 0·04% indole. Control experiments were carried out with untreated sheep and the period of exposure was adjusted so that, if possible, about five egg batches were laid on the controls. Thus in 1937, this took place in 2 hr. during mid-summer, but in September it was necessary to leave the sheep 24 hr. in the field. With sheep that have been treated with a repellent and then made attractive, as a rule no eggs are laid for the first few days; subsequently small numbers of eggs are laid and eventually the flies oviposit freely. The repellent action was regarded as having terminated when more than one egg cluster was found if the controls were severely blown, or when eggs were present at all if few egg clusters were present on the controls. In each test two sheep were used; each animal was lightly sprayed over the back with 600 ml. of an emulsion of the repellent.

Table 2 shows the results obtained. Substances having a pronounced odour, such as clove oil and tagetes oil, did not prove successful repellents. Tagetes oil was recommended by Mönnig (1936) as a blow-fly repellent as a result of chemotropic tests in the laboratory, but this method takes no account of the persistence of the repellent in the fleece. This oil imparted a very strong smell to the fleece but it was noticed that the smell disappeared within

TABLE 2

	Duration of repellent effect in days				
	Sprayed with			Dipped with	
Substance	1%	3%	10%	0·5%	0·8%
Oil of tar:					
Sample A	<3	10	14	—	—
Sample B	<3	5	10	—	—
Sample C	—	6	10	9	—
Clove oil	<3	8	12	—	—
Margosa oil	—	8	—	—	—
Tagetes oil	—	<5	—	—	—
Rape oil	—	5	13	—	—
Olive oil	—	10	16	—	—
Cotton-seed oil	—	12	16	15	—
Castor oil	—	11	—	—	—
Linseed oil	—	9	—	—	—
Soya-bean oil	—	11	—	—	—
Whale oil	—	7	—	—	
Oleic acid	5	12	17	—	11
Kerosene	—	6	—	—	—
p-Dichlorbenzene	—	5	7	—	—
o-Dichlorbenzene	—	5	—	—	—

a few days. Better results were obtained with vegetable oils such as olive oil and cotton-seed oil; their effect may have been due to the development of rancidity as oleic acid proved an excellent repellent. It was found that oleic acid severely irritated the skin and is therefore unsuitable for incorporation in dips or sprays. Various samples of oil of tar gave quite different results; this is not surprising since material from different sources varies considerably in appearance and smell. Sample A which gave good protection was dark in colour and had a strong tarry smell; samples B and C were more refined and had poor

repellent properties. The most promising of the materials tested seem to be cheap vegetable oils such as cotton-seed oil and castor oil.

Discussion

The experiments suggest that repellents may act in various ways. Volatile compounds emit an odour which repels the fly but substances of this type are unsuitable for sheep blow-fly control as a volatile repellent will not persist in the fleece. Non-volatile substances which are either toxic or repugnant to the fly may also exert a repellent effect. A gravid female fly does not immediately lay eggs in the fleece after being attracted to a sheep. Before oviposition begins, there is a preliminary period of excitation lasting several minutes, during which the fly extends its proboscis and appears to feed on the wool. It is feasible to suppose that the fly may be driven away without ovipositing if the wool is coated with a substance which is either toxic or repugnant to the taste or touch. Repellents might be utilized for sheep maggot-fly control in the following ways: (1) by incorporation in dips such as arsenicals to increase their efficiency; (2) by incorporation in maggot dressing for preventing restrike; (3) as sprays for protecting sheep against attack.

With regard to dips, the existing arsenic dips give fair protection for about 3 weeks. Table 2 shows that, even at the high concentrations of repellents used in the dipping experiments, the effect scarcely lasted 2 weeks. The method of testing is extremely critical as a highly attractive material was used; nevertheless, it seems unlikely that a repellent added to the dip would counteract the natural sources of attraction arising in the field for more than 3 weeks at the outside. It may be concluded, therefore, that the effectiveness of the present dips cannot be prolonged by the addition of any of the repellents so far tested. The addition of repellents to maggot dressings has been suggested to prevent the wound becoming reinfested with maggots. A maggot wound is highly attractive to *L. sericata* but the disinfectant present in the dressing usually counteracts this for a few days until the wound begins to heal. Restrike often occurs 2 or 3 weeks later when the scab begins to lift since bacterial decomposition is very liable to occur if the weather is wet. This difficulty might be prevented to some extent by incorporating a repellent in the dressing, but again there is the drawback that the protective effect does not last sufficiently long. The chief value of repellents is probably as sprays for protecting sheep over a short period. The method of spraying recommended by Miller (1935) should be a suitable means of applying a repellent; the sheep are penned and sprayed in groups with a fine mist which settles on the outside of the fleece. Owing to the economy in material, it would be practicable to use the high concentration of repellent necessary to give protection. This means of control is only suitable for lowland sheep which are frequently penned for inspection.

The main object of the work on larvicides was to discover a substance which would protect sheep for a longer period than do the existing sheep dips. Moore (1937) has suggested that the present sheep dips fail because they are water-soluble and therefore readily washed out by rain; he recommended the use of oil-soluble chemicals which could be applied in the form of emulsions, the idea being that the oil would form a film over the wool fibres and skin. This hypothesis is an attractive one and considerable attention was therefore paid to oil-soluble larvicides; synthetic products of this type are now available and several of these were tested as dips. With the exception of dodecyl rhodanate at a high concentration, all the organic larvicides proved inferior to a proprietary arsenic sulphur dip,

although their initial toxicity was about the same as that of arsenic. The main reason for the failure of these organic compounds was due to the fact that they did not persist for long in the fleece. Sheep maggots feed on or close to the skin and it is necessary therefore to maintain a film of poison in this region. The poison must be stable for several weeks at a temperature of about 37° C. when exposed in a thin film and it seems probable that most organic compounds will either volatilize or decompose under these conditions.

Lennox (1938) showed with Merinos that the wool yolk migrates along the fibre away from the skin. The yolk consists of wool grease and a water-soluble fraction, the suint; oil-soluble compounds introduced into the fleece would probably dissolve in the grease, water-soluble compounds in the suint. Work in progress at Bangor indicates that the termination of immunity following an arsenic dip is not due to leaching by rain but to migration of the arsenic away from the skin owing to the growth of new wool and to the movement of the yolk along the fibre. The same limitation would apply to an oil-soluble substance; experiments are in progress to determine whether longer protection is afforded by substances insoluble in oil and water such as the insoluble arsenic salts.

Compounds of this type were included in the dipping experiments described in Table 1. Lead arsenate was only moderately effective probably because its toxicity is considerably lower than the derivatives of arsenious oxide. The arsenites of lead and calcium gave results very similar to the proprietary arsenic dip at an equivalent arsenic concentration. In the case of the insoluble arsenicals, a wetting agent was added to the dip. Subsequent work has shown that the protective properties of soluble arsenic dips are considerably reduced by the presence of a wetting agent in the dipping fluid (Hobson, 1939). When numbers of sheep are dipped, suint accumulates in the bath and imparts good wetting properties to the dip; this results in the sheep dipped last receiving poorer protection against maggot infestation, and probably explains why fly dips often fail when large numbers of sheep are dipped without changing the contents of the bath. In view of this recent work on the effect of wetting agents, the results obtained with insoluble arsenicals assume more importance and further work is in progress with this type of larvicide. It may be noted that McCulloch (1937) showed that calcium arsenite gives good protection against sheep blow-fly attack in Australia, the material being applied by jetting (spraying a suspension into the fleece under pressure).

Summary

1. An account is given of experiments on the protection of sheep against maggot infestation by chemical means.

2. The repellent properties of a number of substances were tested under field conditions. Vegetable oils gave the most promising results, but no repellent was found which protected sheep for more than about 2 weeks.

3. Various larvicides were tried as sheep dips, with a view to finding a substitute for arsenic. None of the substances tested proved superior to a proprietary arsenic-sulphur powder dip. Encouraging results were obtained with insoluble arsenites and further work is in progress with these compounds.

I am indebted to the Agricultural Research Council for a grant which has mainly financed this work. My appreciation is also due to the Imperial Chemical Industries, Ltd., for an additional grant and for supplying certain of the insecticides used in this work.

REFERENCES

DAVIES, W. M. & HOBSON, R. P. (1935). Sheep blow-fly investigations. I. The relationship of humidity to blow-fly attack. *Ann. appl. Biol.* **22**, 279.

HOBSON, R. P. (1935). Sheep blow-fly investigations. II. Substances which induce *Lucilia sericata* Mg. to oviposit on sheep. *Ann. appl. Biol.* **22**, 294.

—— (1936). Sheep blow-fly investigations. III. Observations on the chemotropism of *Lucilia sericata* Mg. *Ann. appl. Biol.* **23**, 845.

—— (1937). Sheep blow-fly investigations. V. Chemotropic tests carried out in 1936. *Ann. appl. Biol.* **24**, 627.

—— (1939). Effect of suint on sheep dips. *Nature, Lond.*, **144**, 1093.

LENNOX, F. G. (1938). Fleece investigations. Paper No. 3. A note on the migration of yolk along wool fibres. *Pamphl. Coun. sci. industr. res. Aust.* no. 83, p. 25.

MACLEOD, J. (1937). The experimental production of cutaneous myiasis of sheep. *Parasitology*, **29**, 526.

—— (1938). On the protection of sheep from maggot-fly. *Bull. ent. Res.* **29**, 149.

MCCULLOCH, R. N. (1937). The present position in blow-fly control in New South Wales. *J. Aust. Inst. agric. Sci.* **3**, 129.

MILLER, W. M. C. (1935). Some observations on the prevention of blow-fly attack in sheep. *Scot. J. Agric.* **18**, 226.

MÖNNIG, H. O. (1936). A new fly repellent and a blow-fly dressing. *Onderstepoort J. Vet. Sci. anim. Ind.* **7**, 419.

MOORE, W. (1937). The chemical control of the sheep maggot fly (*Lucilia sericata* Meigen). *Scot. J. Agric.* **20**, 227.

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GALL MIDGES (CECIDOMYIDAE) AFFECTING GRASS-SEED PRODUCTION IN MID-WALES AND WEST SHROPSHIRE, TOGETHER WITH DESCRIPTIONS OF TWO NEW SPECIES[1]

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Since the establishment of the Welsh Plant Breeding Station at Aberystwyth, grass-seed production has become an important industry in certain parts of Cardiganshire, Montgomeryshire, Shropshire and Herefordshire. In these localities pedigree strains of grasses released by the Station are grown, and the gall midges which affect seed production in the important agricultural grasses are therefore of special interest.

Barnes (1931) and Tomaszewski (1931) catalogued the gall midges known to affect seed production in grasses up to 1931. Since then, Metcalfe (1933) described two new species, *Dasyneura dactylidis* and *Contarinia lolii*, and also claimed to have seen female midges ovipositing on heads of *Bromus* sp. and *Avena* sp. Jones (1936) published a preliminary list of grass gall midges of the mid-Wales area and indicated the existence of many species hitherto unrecorded. Barnes (1939) described two additional species, *Dasyneura festucae* and *D. triseti* and (1940) *Sitodiplosis dactylidis* and *Stenodiplosis geniculati* Reuter var. *dactylidis*.

The present investigation was commenced in 1934 with the object of recording the various species and their host grasses, noting their distribution and obtaining more information concerning their biology, abundance and economic importance. No experimental work on control measures was undertaken. With increased knowledge of the biology of these gall midges, it was possible, however, to suggest certain modifications of cultural practices which could be expected to have an ameliorating effect. These suggestions have been summarized by Evans & Jones (1936). Owing to the difficulty of breeding the adult gall midges, it was not always possible to obtain sufficient material (particularly of the males) to give descriptions of all new species encountered. The characters of the female and larva enabled a few species to be referred to particular genera. In some cases, however, even this partial identification was not possible; in such cases the species is referred to below as "unidentified"; whenever this term is used, it is implied that the species concerned is almost certainly distinct from any known species of grass gall midge. In the present paper the injurious gall midges are serially numbered (the predaceous species are unnumbered) and all are dealt with under the grasses which are arranged alphabetically. Slides of all the new and undescribed gall midges have been deposited in the Barnes collection of gall midges. Drawings illustrating their distinguishing characters are to be found in the writer's thesis.

Alopecurus pratensis L.

Alopecurus pratensis (meadow foxtail) is a valuable agricultural grass. It is commonly found in good pastures and hay fields, but although its use is widespread it is not always abundant. The harvesting of meadow foxtail presents certain difficulties owing to the long flowering period and consequent irregular ripening of the seed; midge attacks, therefore

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in conjunction with the above factor can readily make the commercial production of meadow foxtail seed unprofitable.

Barnes (1930) has constructed a key to the Cecidomyid larvae occurring on the flower heads of meadow foxtail and the following key is based upon it. The colour terminology is derived from the Ridgeway chart.

A. With spatula.
- B. Deep chrome to cadmium yellow; spatula with rounded points; cuticle smooth; posterior spiracles elevated on protuberances of the body wall which project laterally to the anal segment. ... ... **Contarinia merceri** Barnes (1)
- BB. Cadmium orange; spatula with sharp points; cuticle covered with hemispherical warts or papillae; posterior spiracles not elevated. **Dasyneura alopecuri** (Reut.) (2)

AA. Without spatula.
- B. Light ochreous salmon to light ochreous buff to warm buff; no pseudopods; cuticle not bearing long setae. ... ... **Stenodiplosis geniculati** Reut. (3)
- BB. Larvae blood red; pseudopods on ventral surface; cuticle armed with long setae or spines. ... ... **Lestodiplosis** sp.

The writer has observed the occurrence of *Contarinia merceri* in considerable numbers in mid-Wales; *Dasyneura alopecuri* and *Stenodiplosis geniculati* also occur within the area, but no predatory larvae, either of *Lestodiplosis* or of other genera, have been found on meadow foxtail.

(1) Contarinia merceri *Barnes*

This species was first described by Barnes (1930) who gave an account of its biology and also suggested certain control measures. It was first recorded from Cardiganshire by Barnes (1930) who reared the adults from meadow foxtail flower heads forwarded by the provincial advisory zoologist, Mr J. R. W. Jenkins. In mid-Wales, *Contarinia merceri* is the most abundant of all the grass midges and for this reason is the species around which the greater part of the present investigation has centred. The following observations are intended, in the main, to be supplementary to those of Barnes (1930). All the observations on the adults were made at Aberystwyth in 1935 and 1936.

Flight period. In 1935 the first adults were observed on 4 June and were moderately abundant a few days later. The maximum abundance occurred on or about 18 June and the last individuals were observed on 29 June. In 1936 the adults were first seen on 2 June but as they were already fairly abundant on this date it would appear that emergence must have commenced at least 2 or 3 days earlier. The females were observed ovipositing in considerable numbers from 5 to 20 June but no definite period of maximum abundance could be detected during the course of the month. The adults were last seen on 28 June.

Sex ratios. The study of the sex ratio in field populations of *C. merceri* is complicated by the fact that at certain times more or fewer of the females are ovipositing on the flower heads, the remainder of the females and all the males remaining near the ground. At such times it is hardly possible to obtain a fair sample of the population from which the sex ratio can be deduced. However, a certain amount of information can be obtained by studying the fluctuations in the sex ratio among those midges remaining near the ground level. During the present investigation, midges were caught in this situation by means of a brush moistened with 70% alcohol, and were then transferred to a tube for subsequent examination. Preliminary trials showed that there was no discrimination between the sexes.

When, owing to unfavourable conditions, there was no oviposition, the proportion of males was as low as 10–15% at about 6 a.m. Later, it increased steadily until a maximum of about 50% was attained at noon. Thereafter, the proportion of males decreased steadily. The mean percentage of males under such conditions was $21{\cdot}0 \pm 4{\cdot}0$. This is the best estimate of the average sex ratio in a

field population. Since the longevity of the male is approximately equal to that of the female, this ratio should also represent the ratio of male emergences to female emergences during a 24 hr. period or during two or more whole days. Barnes (1930), by the analysis of 2495 emergences of *C. merceri*, showed that the sex ratio is about 24 : 76, which agrees fairly closely with that given above.

The steady increase in the proportion of males among the midges at ground level during the forenoon is a regular occurrence when conditions are such as to prevent oviposition. On one occasion a strong migration of females from the flower heads down to ground level was insufficient to mask the increase in the proportion of males. This increase is probably due to the fact that the males have an emergence crest just before noon (Barnes, 1930). The subsequent decline in the proportion of males can similarly be explained as being due to the emergence of the females, the crest of which occurs shortly after noon (Barnes, 1930).

Migrations. On a relatively calm night during the flight period females of *C. merceri* may be seen in swarms just above the flower heads in an infested meadow foxtail plot. Each swarm, as a whole, tends to remain more or less constant in position. Under such conditions no females have been observed leaving a plot of meadow foxtail. There is certainly no mass migration. On the other hand, females which have been captured in a meadow foxtail plot and released a considerable distance away will not tend to remain in one place but, provided there are no air movements, their flight does not follow any particular direction and is apparently quite unrelated to the surrounding flora. It may be said, therefore, that when there is a number of host plant inflorescences available there is little or no "voluntary" migration. In the absence of such inflorescences, there is a certain amount of voluntary migration of a purely random nature.

Under windy conditions, the females generally take shelter near the ground. The critical wind velocity appears to be about 4–5 m.p.h. As the wind velocity rises, the midges become increasingly helpless. Normally, they will not forsake the shelter of the lower leaves if the wind is strong, but if disturbed they take flight and are immediately swept away. If the wind rises while they are ovipositing on the flower heads, they cling to the florets and resume oviposition only when the wind has dropped. It seems probable that a gusty wind would remove more midges than a strong but continuous wind, though this has not been confirmed. If the vegetation or surface soil to leeward of an infested plot be examined during a windy evening, a considerable number of females can be observed but none can be found on the windward side. It is very difficult to estimate accurately the proportion of midges carried away by a strong wind but the observer is left with the impression that it is by no means insignificant.

The males fly relatively infrequently. Since the females are fertilized soon after emergence, migrations of the males are of little significance in the distribution of the species.

Reactions to stimuli. The following substances, known to be attractive to one or more species of Diptera, have been tested as attractants for the adults of *C. merceri*; alcohol (70%), cedar-wood oil, creosote oil, clove oil, xylol, Lefroy's anti-furniture-beetle fluid (mixture of paradichlorbenzene and raw linseed oil), sugar solutions of various strengths, and water. The sugar solutions were slightly attractive but the other liquids gave negative results, although numbers of other insects were attracted.

The female midges were observed to react negatively to the light of an electric torch and to that of a paraffin lamp. This observation tends to throw some doubt upon the efficacy of bonfires as traps for grass gall midges (Barnes, 1930).

Longevity. As it is impossible to ascertain directly the longevity of midges in the field under natural conditions, observations were made under a range of conditions in cages in the laboratory and in glass tubes in the field. The general conclusion was that the average longevity of both males and females was about 2 days, though some individuals lived for a longer period. The great fluctuations in the sex ratio noted under certain circumstances are also a strong indication of the brief duration of the adult phase.

Oviposition. Detailed results have been prepared for publication elsewhere.

Intra-floral larval phase. The larvae of *C. merceri* were observed within the florets of meadow foxtail at Aberystwyth from the second week in June until the end of the third week in July: up to seventeen larvae were found in a single floret. The method of feeding is somewhat uncertain. When infested florets in the flowering stage (i.e. before the seed has set) are examined, no gross injury to the ovary or stamens can, as a rule, be detected, although when numerous larvae occur in one floret the anthers often become black and apparently diseased. Moreover, even when three to six larvae are present within the floret, the seed is sometimes set and may develop to a limited extent without exhibiting signs of gross injury.

Migration of larvae from flower heads. When full grown the larvae emerge from the florets and migrate to the ground. If the infestation is sufficiently intense, numbers of larvae can be seen emerging from the florets after a shower of rain. Such observations were frequently made by the writer in June 1936. The larvae could be seen slowly wriggling their way out between the glumes, the operation sometimes occupying as much as an hour. The majority of the larvae came out hinder-end first. It was noticeable that a superabundance of water on the grass heads greatly retarded the movements of the larvae; under such conditions they appeared to be held by the surface film and could reach the soil only by crawling slowly down the flower shoot. Under drier conditions, the larvae progressed more rapidly and usually "jumped" to the ground.

It has also been observed that a considerable number of larvae emerge during the night after a moderate dew. Thus, to take a particular case, after a dry day on 26 June, 1936, dew began at approximately 10.30 p.m.; no larvae were observed at this time but at 3.30 a.m. (no rain had fallen in the meantime) twenty-four larvae were counted on 100 flower heads; the emergence and migration of the larvae continued until 6 a.m. at which time the observations were discontinued. A simple experiment performed in the field but under cover (to exclude rain) provided confirmation of the conclusion that dew provides sufficient moisture to enable the larvae to escape. A bunch of meadow foxtail flower heads with long stalks were arranged with the stalks dipping into water in a narrow-necked vessel, the neck being stopped in cotton-wool which was not allowed to come into contact with the water. The whole was placed on a large sheet of white paper. Every night for a week a number of larvae (10–40) emerged from the heads and were collected from the paper in the morning.

Soil relations of the larvae. Having attained the ground level, either by jumping or by crawling down the grass stems, the larvae make their way between the soil particles until they reach a suitable depth where, after a time, cocoons are formed. The larvae remain within these cocoons throughout the winter and until late in the following spring. According to Barnes, the larvae leave their cocoons in the spring and wander about in the soil during May. Then pupation takes place without the formation of another cocoon just over a week before the adult midge emerges. The pupae make their way upwards to the surface before the adults emerge.

In view of the possibility of controlling the midges by soil treatment, ploughing or inter-drill cultivation, the depth distribution of the larvae is a problem of some importance. Observations in a drilled plot showed that $95{\cdot}4 \pm 1{\cdot}6\%$ of the larvae occurred in the upper inch. The remainder occurred in the second inch. The vast majority, therefore, are very near the surface of the soil. No broadcast plots were available for observation. On two occasions, soil samples which had not been examined within a day of the date of collection were set aside for several days in a well-broken condition. The resulting desiccation did not appear to affect the larvae. Moreover, there was no indication that, under such unfavourable conditions, the larvae could emerge from their cocoons to seek more favourable situations. The enormous number of larvae which overwinter in the soil in a heavily infested plot may be gauged from the following observation. Five soil samples, each with a surface area of 3·8 sq. in., gave a mean number of larvae of $53{\cdot}8 \pm 4{\cdot}8$; this is equivalent to about 2000/sq. ft.

(2) Dasyneura alopecuri (*Reuter*)

This species was described by Reuter (1895) as *Oligotrophus alopecuri*. Barnes (1927) described as a separate species *Dasyneura agropyronis*, a midge found swarming on *Agropyron repens* Beauv. (couch-grass). Later (1930) he identified this species with *Oligotrophus alopecuri* Reut. which he transferred to the genus *Dasyneura* Rond, and the biology of which he described in some detail. This species appears to be extremely rare in mid-Wales where it is of no economic importance.

(3) Stenodiplosis geniculati *Reuter*

This species was first described by Reuter (1895) who recorded it from *Alopecurus geniculatus* L. (marsh foxtail). Barnes (1930) bred the adults from *A. pratensis* L. (meadow foxtail) collected in various localities of Britain and gave accounts of the biology and dis-

tribution. The insect is on the whole extremely rare in mid-Wales and has been recorded only from Montgomeryshire and Shropshire, and only on *A. pratensis* L. Jones (1936) recorded an infestation of 12–15 % in a few small samples.

Arrhenatherum avenaceum Beauville

Arrhenatherum avenaceum Beauv. (tall or false oat-grass) is common throughout Britain. It has a certain limited use in agriculture, particularly in short leys. Hitherto, only one species of Cecidomyid, viz. *Contarinia arrhenatheri* Kieffer, has been recorded from this grass. The writer has observed this species in mid-Wales and, in addition, the larvae of six other species have been recorded, two being predatory. The following key suffices for the separation of all seven species:

A. Pseudopods on ventral surfaces of mesothorax, metathorax and first seven abdominal segments; anal segment with a pair of biarticulate appendages. ... **Arthrocnodax** sp.

AA. Without the above pseudopods and bi-articulate appendages.

- B. Sternal spatula present.
  - C. Spatula head bilobed.
    - D. Cuticle conspicuously and almost completely covered with scales.
      - E. Anal segment with pair of small tubercular processes but without papillae armed with stout conical processes or long setae. **Bremia** (sens. lat.) sp.
      - EE. Anal segment without tubercular processes but with two pairs of papillae armed with stout conical processes, one pair with long setae and one pair with minute setae. ... Unidentified species (6)
    - DD. Cuticle smooth except for a few rows of scales near the anterior margin of each segment; posterior spiracles not elevated. **Clinodiplosis** sp. (5)
    - DDD. Cuticle smooth except for a few rows of scales on the anal segment; posterior spiracles elevated on processes of the body wall. **Contarinia arrhenatheri** Kieffer (4)
  - CC. Spatula head unidivided, transverse, membranous. ... Unidentified species (7)
- BB. Sternal spatula absent. ... ... Unidentified species (8)

(4) Contarinia arrhenatheri *Kieffer*

The female only was described by Kieffer (1901). Barnes (1931) records the capture of a single female in Hertfordshire, June 1928. The species is widely distributed in mid-Wales but is not very abundant, although a few infestations of 10–25 % have been recorded in Montgomeryshire. It is of no economic importance.

(5) Clinodiplosis *sp.*

The larvae of this species may be inquilines. It has not been possible to relate floret injury to their presence. They have been found only in Montgomeryshire.

(6, 7, 8) *Three unidentified species*

The larvae of these species are phytophagous. Those of (6) have been found only at Chirbury, Shropshire. The others are more widely distributed but are by no means common. The larvae of all three species were found in the florets of tall oat-grass in July.

Bromus sterilis L.

Bromus sterilis L. (barren brome-grass) commonly occurs in hedgerows and waste places. It is of no agricultural value. Metcalfe (1938) observed female gall midges ovipositing on *Bromus* sp. The writer has frequently found Cecidomyid larvae in the florets of *B. sterili*- but not in those of other species of *Bromus*.

(9) Contarinia *sp.*

The larvae of this species are distinguishable from those of other species of *Contari*- occurring on grasses by the extensive granulation of the cuticle. Its life history appe to be of the normal grass *Contarinia* type. It has been recorded only from Montgomerysh where the host grass occurs in abundance.

Dactylis glomerata L.

Dactylis glomerata L. (cocksfoot) is an important agricultural grass and is used extensiv both for hay and pasture. Two species of Cecidomyidae, viz. *Contarinia dactylidis* (H.L and *Dasyneura dactylidis* Metcalfe, have previously been recorded on cocksfoot; Bagnall Harrison (1918) record "Cecidomyid larvae" in cocksfoot spikelets. Jones (1936) dr attention to the occurrence of a third species on this grass in mid-Wales. In addition, t predatory larvae of a species of *Lestodiplosis* have also been found on cocksfoot in this ar The following key facilitates the separation of the larvae of the four species:

A. With spatula; without pseudopods.

B. Yellow to orange; cuticle smooth; posterior spiracles situated on backwardly projecting protuberances of the body wall; spatula "points" rounded. **Contarinia dactylidis** (H.Lw.) (10)

BB. Pale red to pink; cuticle granulated; posterior spiracles not elevated, relatively inconspicuous; spatula with sharp points. ... **Dasyneura dactylidis** Metcalfe (1

BBB. Orange and red; cuticle almost completely covered with bluntly pointed scales or papillae; posterior spiracles not elevated, not unusually conspicuous; spatula points rather sharp. ... ... **Sitodiplosis dactylidis** Barnes (1

AA. Without spatula; with pseudopods. ... ... **Lestodiplosis** sp

A single larva of a fourth (phytophagous) species (13) was dissected from a cocksfo seed from a sample collected near Montgomery. This larva has a general resemblance a *Dasyneura* larva but, although of a large size and therefore presumably full grown, possesses no sternal spatula. There are also other slight differences. Barnes (1940) conside that this is a specimen of *Stenodiplosis geniculati* Reuter.

(10) Contarinia dactylidis (*H.Lw.*)

The original description of this species (H. Loew, 1851) has been amplified by Metcal (1933) who also gives a brief account of its biology, including notes on certain immuni trials performed at Harpenden.

According to the present writer's observations, the life-cycle of *Contarinia dactylidis* is essentially the same as that of *C. merceri*. At Aberystwyth in 1935, the flight period as observed in the field extended from about 4 June to 2 July with a maximum prevalence about 18 June. In 1936 the emergence extended over the same period but there was no

definite maximum. The oviposition behaviour closely resembles that of *C. merceri* and shows a tendency towards maximum activity in the late evening and early morning. Adults caught in the field at a time when there was no oviposition gave a sex ratio of 36 : 64 (males : females). Metcalfe (1933) states that the "larvae...feed collectively, five or six to the flower", but this is not substantiated by the present writer's observations. As in *C. merceri*, the migration of the larvae to the soil occurs only under conditions of high humidity.

In the mid-Wales area, old cocksfoot stands are liable to be heavily infested by *C. dactylidis*. Blindness directly due to the larvae of this species has been known to occur in as many as 60% of florets. Infestations of 15–30% are more common, however. *C. dactylidis* must be regarded as a serious pest in the cocksfoot seed-producing areas of mid-Wales and Shropshire.

(11) Dasyneura dactylidis *Metcalfe*

The description of this species and an account of its biology are given by Metcalfe (1933). It is of no economic importance in mid-Wales, the highest infestation recorded so far being only 3% of florets. Unlike the larvae of *Dasyneura alopecuri* on foxtail, those of *D. dactylidis* do not remain within the florets but, when full grown, migrate to the soil for overwintering and pupation. They readily emerge from stored seeds even when the latter are not deliberately moistened.

(12) Sitodiplosis dactylidis *Barnes*

No males of this species, which has recently been described by Barnes (1940), have been obtained. The female and the larva resemble those of the species of *Sitodiplosis* (20) on *Poa trivialis* and *P. pratensis*.

The species is fairly widely distributed in mid-Wales and is sufficiently abundant to be regarded as a potentially serious pest of cocksfoot. Infestations up to 20% have been recorded. The larvae, which are frequently parasitized, generally occur singly in the florets and feed at the base of the developing seed. When full grown they migrate to the soil.

(13) Stenodiplosis geniculati *Reuter*. *Single larva only*

Festuca pratensis Hackel and *F. rubra* L.

Both meadow fescue (*Festuca pratensis*) and red fescue (*F. rubra*) are important agricultural grasses. Barnes (1931) recorded the occurrence of Cecidoymid larvae in the flower heads of *F. rubra* var. *arenaria* collected in Oxfordshire and has recently (1939) described the species under the name *Dasyneura festucae*. The present writer has not observed this species in mid-Wales but another species, described below, has been found on both *Festuca pratensis* and *F. rubra*.

(14) Contarinia festucae *n.sp.*

Male. Body length 1·1–1·6 mm. Antennae: 2 + 12; fuscous brown; of typical *Contarinia* structure; basal and distal nodes of third flagellar segment about equal in size; stem and neck of third flagellar segment about equal in length and about $\times 3$ as long as broad; stem about $\times 1\frac{1}{4}$ as long as the basal node; stem and neck of tenth flagellar segment $\times 3\frac{1}{2}$–5 as long as broad; distal elongation of twelfth flagellar segment about $\times \frac{2}{3}$ the length of the stem; each node with a whorl of 8 or 9 looped regular circumfila, arising distally and approximately equalling in length the stem or neck; each node also with a whorl of regular setae about $\times 1\frac{1}{4}$–2 the length of the circumfila. Palps: pale yellow; sparsely setose; basal segment quadrate, second $\times 1\frac{1}{2}$ as long as the first, third $\times 2\frac{1}{4}$ as long as the first, fourth about $\times 2\frac{3}{4}$ as long as the first; basal palp segment about $\times 1\frac{3}{7}$ as long as broad, second $\times 2$ as long as

broad, third about $\times 3\frac{1}{2}$ as long as broad, and fourth about $\times 4\frac{4}{5}$ as long as broad. Face: fuscous. Eyes: black. Thorax: dark brown dorsally, pale brown laterally and ventrally. Wings: hyaline. Legs: yellow with fuscous hairs. Abdomen: pale brown to yellow. Claws: simple. Empodium: equal in length to claws. Genitalia: basal clasp segment fuscous brown, with long setae; distal clasp segment of a lighter brown, without setae; dorsal plate deeply bilobed, the lobes rounded in general form but slightly triangulated at the apex; ventral plates deeply bilobed, the lobes smoothly rounded; style rounded, almost invariably very slightly shorter than ventral plate.

Cotypes: Cecid. 4191–3 in the Barnes collection.

Female. Body length: rather longer than in the male. Antennae $2+12$; of typical *Contarinia* structure; first flagellar segment about $\times 1\frac{1}{2}$ as long as the second, $\times 4$ as long as broad, $\times 7$–8 as long as the neck; third flagellar segment about $\times 2\frac{1}{3}$ as long as broad, nearly $\times 4$ as long as the neck. Palps: basal segment quadrate, second $\times 1\frac{1}{2}$ as long as the first, third $\times 2\frac{3}{10}$ as long as the first, fourth nearly $\times 3$ as long as the first; first, second, third and fourth segments, $\times 1\frac{1}{5}$, $\times 1\frac{2}{3}$, $\times 3\frac{2}{7}$, $\times 4\frac{2}{3}$, respectively, as long as broad. Abdomen pale brown. Ovipositor: aciculate, very long and slender. Otherwise similar to the male.

Cotypes: Cecid. 4194–6 in the Barnes collection.

Larva and *pupa*: practically indistinguishable from those of most other species of *Contarinia* occurring on grasses.

Larvae: Cecid. 4197–9 in the Barnes collection.

C. festucae can be distinguished, with greater or less difficulty, from *C. tritici*, *C. avenae*, *C. merceri*, *C. dactylidis*, *C. lolii* and *Contarinia* sp. on *Poa pratensis* and *P. trivialis* (the last-named described by Tomaszewski, 1931) by the combination of characters described above, particularly the number of circumfila loops and the relative proportions of the palp and antennal segments. The description of *C. brizae* given by Kieffer (1896) is extremely brief but the colour features recorded are quite different from those of *C. festucae*. Only the female of *C. arrhenatheri* has been described (Kieffer, 1901); here again the description is inadequate but the relative proportions of the palp segments, at least, are different. Important morphological differences separate *C. festucae* from the extra-European species of *Contarinia* infesting grasses, viz. *C. sorghicola*, *C. caudata* and *C. andropoginis*.

The flight period of *C. festucae* appears to be somewhat earlier than that of *C. merceri* and *C. dactylidis*. At Aberystwyth in 1936, the adults were observed from 21 May until 15 June. 122 adults bred in the insectary gave a sex-ratio of 21 males : 79 females which approximates to that in *C. merceri* (see above).

Up to eight larvae may occur in a single floret but as a rule only one or two are found. The period of seed infestation, as shown by the examination of seed samples in 1935 and 1936, is over by the end of the first week in July. In this respect, again, the species is definitely "earlier" than either *C. merceri* or *C. dactylidis*. The larvae migrate to the soil when full grown. Examination of soil samples from an infested plot showed that the larvae over-wintered in the upper inch of soil. A striking feature of the over-wintering larvae was the fact that by the beginning of April only a very small proportion (about 8%) had formed cocoons. By the end of April the proportion had risen to about 37%. For the purpose of comparison between *C. festucae* and *C. merceri*, it should be mentioned that the above observations relating to *C. festucae* were made in a broadcast plot, whereas in the case of *C. merceri* the observations were made in a drilled plot. Pupation occurred a few days before emergence. Parasitism was common.

Up to the present *C. festucae* has been observed in only one seed-production plot. In this case the seed infestation was about 18%. An infestation of about 14% has been recorded in *Festuca pratensis* growing wild. The species is, therefore, potentially an

important pest of *F. rubra* and *F. pratensis* and possibly of other fescues. It has been found only at Aberystwyth.

Holcus lanatus L.

Holcus lanatus L. (Yorkshire fog) is widely distributed and very common in mid-Wales. It has some slight agricultural value, though often regarded with disfavour. The larvae of four species of Cecidomyidae have been recorded on this grass. The larvae of the last species listed in the following key are predatory.

A. Spatula present; pseudopods absent.
- B. Yellow; cuticle without spines dorsally. ... ... **Contarinia** sp. (15)
- BB. Orange-red and red; cuticle densely granulated. ... ... **Dasyneura** sp. (16)
- BBB. Orange and red; cuticle with relatively large spines dorsally. **Sitodiplosis** sp. (17)

AA. Spatula absent; pseudopods present. ... ... **Lestodiplosis** sp.

(15, 16, 17) *Species of* Contarinia, Dasyneura *and* Sitodiplosis

The *Contarinia* larvae have been commonly found in Yorkshire fog seed samples from mid-Wales. Infestations of about 20% have been recorded. The life history appears to conform with that of other species of *Contarinia* infesting grasses. Full-grown, as well as half-grown, larvae have been found feeding in the male florets (certain of the florets are bi-sexual). Only two *Dasyneura* larvae have been found. They are indistinguishable from those of *D. dactylidis* on cocksfoot but this does not establish their identity. The larvae of the third type, provisionally referred to the genus *Sitodiplosis*, are also very rare.

Lolium perenne L.

Lolium perenne L. (perennial rye-grass) is an important agricultural grass. Its seeds are frequently infested by the larvae of *Contarinia lolii* Metcalfe (1933). The larvae of two other species, believed to be predatory, have been recorded by the present writer. The following is a key to the larvae of all three species.

A. Without spatula; with pseudopods. ... ... **Lestodiplosis** sp.

AA. With spatula; without pseudopods.
- B. Yellow; cuticle without scales or flattened spines; posterior spiracles raised on protuberances which project laterally to the anal segment. **Contarinia lolii** Metcalfe (18)
- BB. Orange; cuticle of each segment with a few rows of scales or flattened spines; posterior spiracles inconspicuous, not elevated. ... ... **Bremia** (sens. lat.) sp.

(18) Contarinia lolii *Met.*

A description of the species, together with an account of its bionomics, are given by Metcalfe (1933), who records it as a relatively unimportant pest of *Lolium perenne* at Harpenden, Herts. It also appears to be unimportant in mid-Wales where the highest infestation recorded was only about 5%. The larvae of this species have not as yet been found in the florets of *L. italicum* Braun (Italian rye-grass).

Poa pratensis L. and *P. trivialis* L.

Smooth-stalked meadow-grass (*Poa pratensis*) and rough-stalked meadow-grass (*P. trivialis*) are both important agricultural grasses, the latter being particularly valuable. During the present investigations, *Contarinia* and *Sitodiplosis* larvae were found on both grasses. As the two grasses are very closely related and since no differences could be detected between the larvae of the respective genera obtained from the two hosts it seems highly probable that there is only one species of each genus involved.

The *Contarinia* and *Sitodiplosis* larvae may be separated as follows:

A. Yellow; cuticle smooth, without scales; spatula cleft with an angle of almost 90°; posterior spiracles elevated on protuberances of the body-wall which extend laterally to the anal segment. ... ... **Contarinia** sp. **(19)**

AA. Orange-red; cuticle with scales; cleft of spatula less than 60°; posterior spiracles not specially elevated. ... ... **Sitodiplosis cambriensis** n.sp. **(20)**

(19) Contarinia *sp.*

Von Oettingen (1929) gave a brief account of the biology of an unnamed Cecidomyid infesting the flower-heads of *Poa pratensis* in Germany. Tomaszewski (1931) examined material supplied by von Oettingen and identified it as a species of *Contarinia*. The same author gave a detailed description of the species (based on his own material) but refrained from giving it a name. A considerable amount of biological data relating to this species has been accumulated by von Oettingen (1927, 1929, 1930), Schwarz & Tomaszewski (1930) and Tomaszewski (1931). The life history is that of the normal grass *Contarinia*.

Infestations up to 5% were recorded by the writer in various localities in mid-Wales. The species is therefore not very injurious in this area although in Germany it has been known to produce an almost complete failure in the seed crop (von Oettingen, 1930). It must therefore be regarded as a potentially serious pest.

(20) Sitodiplosis cambriensis *n.sp.*

Male. Palpi consisting of three segments, the basal segment showing indications of further segmentation. Antennae 2+12; the first two segments of the flagellum fused; the first consists of a more elongate, subcylindrical basal node and a shorter, subovoid distal node; the following segments have a shorter subglobular basal node and an elliptical, or subcylindrical distal node; each node carries a single circumfilum with about ten loops, the length of the latter being equal to or slightly less than the breadth of the stem, and a whorl of hairs which are about half as long as the segment and very much longer than the loops of the circumfilum; stem never as clearly differentiated as the neck, more prominent in the segments from the fourth to the tenth than in the others, at most only as long as the basal node; the last segment with a long appendage swollen at its base. Wings hairy, third vein curved, terminating beyond the apex of the wing, fifth bifurcate. Tarsi hairy; claws all simple, about as long as the empodium. Genitalia: basal clasp segment with a median angular process at about the middle of its length; terminal clasp segment strong, curved, glabrous except for some short sparse hairs; dorsal plate divided by a triangular incision into two lobes which are obliquely truncate; ventral plate much longer than the dorsal, almost straight, with a rounded incision apically, and much shorter than the stylet.

Type: Cecid. 4200 in the Barnes collection.

Female. Flagellar segments cylindrical, elongated, gradually shortening, neck equalling three-quarters of the length of the node. Ovipositor moderately extensible, provided with two large lateral lobes and a small ventral lobe. Otherwise about as in male.

Cotypes: Cecid. 4201–5 in the Barnes collection.

Larva. Length about 1·5–2·1 mm.; breadth, 0·3–0·5 mm. Colour orange red, the extreme posterior end almost colourless. Body cylindrical, slightly flattened dorso-ventrally; tapering anteriorly and posteriorly but not attenuated at either end. Cuticle with scale-like warts or papillae dorsally and laterally. Distal antennal joint short, thick. Eye spots dark crimson. Spatula well developed; head deeply cleft, cleft roughly triangular with an angle of 45–50°; the two lobes bluntly pointed and with small lateral lobes projecting (laterally) well beyond the adjacent part of the stem; stem narrowing considerably posteriorly. Dorsal and ventral *verrucae spiniformes.* Eight terminal papillae; one lateral pair with moderately long setae, another pair with very short setae, and two pairs with strong, dark yellow conical processes of which the members of the outer pair at least are slightly hooked. Posterior spiracles near the posterior angles of the penultimate segment; a dorsal papilla armed with a seta immediately medial to each spiracle.

Larvae: Cecid. 4206–7 in the Barnes collection.

Little is known as yet of the biology of this species. The females were observed ovipositing on *Poa trivialis* on the evening of 24 June 1936, and a male and female were caught *in copula.* Mating had apparently commenced at the ground level, and, in this case (which was probably abnormal), the male and female had flown up to the panicle while still united. The larvae were found in the seeds from 29 June to 3 July. On the latter date a number of individuals were moulting and a few were without spatulae. It would appear, therefore, that the period of larval infestation of the flower heads extends until much later in July. The larvae presumably emerge from the seeds when full grown although this has not been observed.

On two occasions an infestation of 10–15% has been recorded. Generally, however, the infestation is very much lower. The species may be regarded as a potentially serious pest. It occurs in both Cardiganshire and Montgomeryshire and is probably much more widely distributed.

Summary

Twenty species of Cecidomyidae are dealt with which affect seed production in the following grasses in mid-Wales and West Shropshire: *Alopecurus pratensis* (3 species), *Arrhenatherum avenaceum* (5), *Bromus sterilis* (1), *Dactylis glomerata* (4), *Festuca pratensis* and *F. rubra* (1), *Holcus lanatus* (3), *Lolium perenne* (1) and *Poa pratensis* and *P. trivialis* (2). Notes on their biology and economic importance are included. Eleven of these species are believed to be new to science and descriptions are given for two, viz. *Contarinia festucae* on *Festuca* spp. and *Sitodiplosis cambriensis* on *Poa* spp. Keys are provided to facilitate the identification of the larvae on the various host grasses.

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REFERENCES

All references prior to Barnes (1931) and Tomaszewski (1931), and included in those papers, are for the sake of brevity omitted from this list.

Barnes, H. F. (1931). Gall midges (Cecidomyidae) whose larvae prevent seed production in grasses (Gramineae). *Bull. ent. Res.* **22**, 199.

—— (1939). Grass-seed *Dasyneura* gall midges, together with the description of two new species. *Arb. phys. angew. Ent.* **6**, 171.

Barnes, H. F. (1940). The gall midges attacking the seed heads of cocksfoot, *Dactylis glomerata* L., with special reference to *Stenodiplosis geniculati* Reuter and its new variety *S. geniculati* var.nov. *dactylidis*. *Bull. ent. Res.* **21**, 111.

Evans, G. & Jones, D. P. (1936). The control of gall midges affecting seed production in grasses. *Welsh J. Agric.* **12**, 198.

Jones, D. P. (1936). Gall midges affecting grass-seed production in mid-Wales. *Welsh J. Agric.* **12**, 192.

Metcalfe, M. E. (1933). Some Cecidomyidae attacking the seed of *Dactylis glomerata* L. and *Lolium perenne* L. *Ann. appl. Biol.* **20**, 327.

Tomaszewski, W. (1931). Cecidomyiden (Gallmücken) als Grasschädlinge. *Arb. biol. Abt.* (*Anst.-Reichsanst.*), *Berl.*, **19**, 1–15.

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STUDIES OF THE BIOLOGY OF THE DEATH-WATCH BEETLE, *XESTOBIUM RUFOVILLOSUM* DE G.

III. FUNGAL DECAY IN TIMBER IN RELATION TO THE OCCURRENCE AND RATE OF DEVELOPMENT OF THE INSECT

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Fisher (1938) directed attention to the scanty information available from literature on the details of the life history and habitat of the death-watch beetle, *Xestobium rufovillosum* De G. Except for occasional mention of its occurrence in decayed parts of certain hardwoods, past workers have paid little attention to the condition of the timber in which the insect has been found or to the factors determining its suitability for infestation by this Anobiid and its rate of development. A knowledge of these factors would obviously be of value for the prevention and control of damage by the death-watch beetle in structural timbers in buildings, and at the same time would throw light upon its food relations, of interest in connexion with the wider problem of the physiology of nutrition of wood-boring insects.

In the course of an investigation of these factors, temperature and humidity have been shown to be of importance (Fisher, 1937). The part played by fungal decay in determining the degree of suitability of timber for infestation by *Xestobium* is the subject of the present paper, which presents the results of field observations and experimental work upon the occurrence and rate of development of this insect in sound and decayed wood. Since the investigation was started its scope has been extended, in co-operation with the Wood Chemistry Section of the Laboratory, to enquire into the effect of fungal decay and subsequent death-watch beetle infestation upon the chemical composition of the wood with a view to ascertaining how, if at all, fungus prepares the way for attack by the insect. The results of this aspect of the investigation will be published separately.

Field observations

Natural habitat

The natural habitat of *Xestobium* is in decayed parts of standing hardwood trees, most commonly oak and willow. Portions of decayed and hollow trunks of old parkland trees, such as many of the oaks in Richmond Park and Windsor Great Park, frequently show evidence of having been attacked, but examination has shown that they do not always contain living insects. It appears that such trees are no longer suitable for attack, and that although decayed wood may be necessary for the insect other factors may affect its degree of suitability. No data are available from specific identifications of the fungi responsible for the decay in these trees, but according to Cartwright & Findlay (1936) the most common cause of heart rot in standing oaks is *Polyporus sulphureus* (Bull.) Fr., and it is probable that at some stage in the progress of decay brought about by this fungus the attacked timber

becomes suitable for infestation by the death-watch beetle. Living larvae and beetles have been found in parts of a newly felled parkland oak showing the typical brown colour produced in the timber by *Fistulina hepatica* (Huds.) Fr. Braid (1924) has suggested an association between the presence of this fungus and the hollow stag-headed condition of oaks, often a feature of old trees, showing evidence of death-watch beetle damage. In its later stages of development this wood destroyer leaves the timber in a harder condition than *Polyporus sulphureus*, and typical of that in which *Xestobium* tunnels are often found in the trunks of old oaks. The suitability of "brown oak" caused by infestation by this fungus in its early stages, for the development of death-watch beetle larvae, has been the subject of experiment in the course of the present investigation.

Willow trees growing along the banks of streams or ditches in Oxfordshire have been a prolific source of supply of living material of *Xestobium*. In all cases the insect was found only in rotted parts of the trees, generally on hollow trunks. Samples of infested wood cut from the trees revealed the presence of wood-destroying fungi, whilst fungi collected from willows in the neighbourhood were *Polyporus fumosus* (Pers.) Fr., *Polystictus versicolor* (Linn.) Fr., *Trametes suaveolens* (Linn.) Fr., and *Coniophora cerebella* Pers. Fruiting bodies and stromatic sheets of mycelium of *Xylaria hypoxylon* Grev. were obtained from one of the trees heavily infested by *Xestobium*. It is noteworthy that the insect did not occur in the most severely rotted, softest portions of the stem, and as in the case of oaks, parts of the decayed timbers frequently contained old tunnels but no living insects, indicating that some condition of the wood apart from the mere presence of decay may be necessary for *Xestobium* infestation.

In buildings

During the past 10 years the Forest Products Research Laboratory has inspected numerous buildings throughout the country and has collected information upon the position and condition of timbers in which serious damage has been caused by *Xestobium*. Damage was invariably most severe in built-in parts of timbers, such as the ends of tie-beams, in wall-plates or other woodwork where ventilation was poor and where, if moisture were present, conditions would be suitable for fungal attack. The decayed condition of such timbers was often apparent from superficial examination but even when not evident, localized patches of insect attack, as shown, for example, by groups of exit holes in panelling or flooring, were usually found to be associated with rotten woodwork in the immediate vicinity. In a few cases evidence of living fungus was obtained and it was possible to identify the species, but in the majority of buildings examined, no signs of fungal activity were noted, the decay having taken place in the past when moisture conditions, brought about, for instance by leaking gutters or defective roof coverings, were suitable.

Whilst these observations show that death-watch damage is most severe in obviously decayed woodwork, they also prove that extensive damage is of frequent occurrence in timbers in which external signs of decay may not be present. When examined microscopically, however, such timber was always found to contain fungal mycelium, although it was not usually possible to identify the species present. Examination of representative samples of death-watch beetle-infested timber from buildings has revealed the presence of a number of fungi of which the following have been identified.

Phellinus cryptarum Karst. This species has been found in association with severe *Xestobium* attack in oak roofing timbers, in the ends of tie-beams, wall-plates, rafters, oak panelling and flooring. In some instances, the fungus was active in the vicinity of beetle-infested wood, but in others it had died out and the insect was working in the decayed woodwork. According to Cartwright & Findlay (1936) it is probably able to cause more rapid decay of oak in buildings than any other species, but it requires very moist conditions and is most likely to cause serious decay in oak roofs where leakage has occurred, or it may be confined to the interior of a beam in a moist condition. Thus, the parts of beams embedded in masonry or brickwork or covered with plaster are most liable to attack. It is in such positions that severe death-watch beetle damage is most often found in oak structural timbers, and it is evident that the presence of this fungus in a building has an important bearing on the occurrence and severity of *Xestobium* damage. This insect was found by Mangin & Patouillard (1922) in a number of oak beams in the Palace of Versailles where *Phellinus cryptarum* had caused extensive decay. The significance of the decay in relation to subsequent infestation by *Xestobium* is illustrated by the resistance shown by the medullary rays, first to the fungus and later to the insect, with the result that they often appear as distinct laminae in the midst of the disintegrated tissues.

Coniophora cerebella Pers., cellar fungus; *Merulius lacrymans* (Wulf.) Fr., dry-rot fungus. Softwoods and hardwoods decayed by either of these fungi have been found attacked by *Xestobium* in buildings. Instances of infestation have been recorded in softwood flooring joists and boards and sometimes in adjacent oak woodwork, in softwood panelling and also in joists and wall-plates of oak. Evidence has been obtained to suggest that in old churches *Xestobium* infestation of floor blocks, pews, panelling, etc., is usually associated with the presence of decay caused by one or other of these fungi, and that the beetle originating from the oak roofing timbers found conditions suitable for its development in timber in other parts of the building where faulty construction and inadequate ventilation had favoured development of fungal decay.

In the days of wooden ships, dry rot was responsible for great damage in naval vessels (Ramsbottom, 1937). No records are available of death-watch beetle damage in these ships, but this insect has been responsible for extensive injury to the main timbers of H.M.S. *Victory* now in dry dock. Although the origin and date of first infestation by the insect are not known, the condition of the timbers and progress of attack have been kept under observation during the past eight years and inspections have shown that the insect is present in timbers which have been severely decayed, chiefly by *Merulius lacrymans*. Ramsbottom, examining the timbers some 6 or 7 years ago, also found signs of a former extensive attack by *Coniophora cerebella*.

Polyporus sulphureus (Bull.) Fr. This fungus, in association with *Merulius lacrymans*, was identified as one of the causes of decay of the ship's timbers mentioned above, in which severe *Xestobium* damage was found. In two other instances *Polyporus sulphureus* was considered the most likely cause of decay of oak joists and other structural timbers in which the insect had caused extensive damage. According to Cartwright & Findlay (1936) numerous cases of decay in oak in buildings can be traced to infection by *P. sulphureus*, which was present in the standing tree. Since any trace of infection can develop so long as the timber remains moist, and in view of the practice in the past of using unseasoned oak of large dimensions which dries slowly, considerable decay could occur before the timber became too dry for

further growth of the fungus. It is conceivable that the presence of this fungus in oak structural timbers of old buildings at the time of their erection has been a factor in rendering the timber suitable for death-watch beetle attack and favouring its spread.

Poria vaillantii (D.C.) Fr. Traces of this species, no longer active, were found rarely in oak and elm attacked by *Xestobium*. It attacks chiefly softwoods and is a common cause of dry rot in buildings where leakage of water has occurred, and can cause extensive decay.

Poria medulla-panis (Pers.) Fr. This species was recorded on one occasion from oak roofing boards beneath a faulty lead roof. Death-watch beetle was active in the immediate vicinity but was not noted in the boards attacked by the fungus: their sodden condition may have rendered the timber unsuitable for attack until they had dried out. In another instance this fungus was suggested as a possible cause of decay in part of an oak structural timber in which *Xestobium* was present. The type of decay caused by this species is similar to that produced by *Phellinus cryptarum*.

Fistulina hepatica (Huds.) Fr. Death-watch beetle damage has been found in oak structural timbers showing the characteristic brown discoloration caused by this fungus in the standing tree.

Paeciliomyces varioti Bainier. A bright golden brown colour sometimes found around death-watch beetle tunnels in oak is regarded by Cartwright & Findlay (1936) as similar to the condition caused by the growth of this mould and to which the name of "golden oak" has been given by Williamson (1923) who called the fungus *Eidamia catenulata*.

In addition to the above fungi others have been noted as being responsible for decay in *Xestobium*-attacked wood, but precise identification of the species has not been possible.

Whilst these observations on the condition of timbers in buildings attacked by the death-watch beetle show that decayed wood is apparently preferred by the insect, they also prove that it is not associated with any particular fungus or specific type of fungal decay. For instance, it occurred in timber which had been attacked by wood-destroying fungi causing white rots, e.g. *Phellinus cryptarum*,[1] where lignin is attacked as well as the carbohydrates, and it was also found in association with decay of the brown-rot type, e.g. *Merulius lacrymans* and *Coniophora cerebella*, where preferential attack is made on the carbohydrate components of the wood substance and lignin is in the main unaffected. Inspections of buildings and examination of samples of death-watch beetle-infested wood from many localities throughout England confirm the opinion to which attention was called by Lefroy (1924) that a characteristic feature of death-watch beetle attack is its cessation before all the available timber has been destroyed. With the object of seeking an explanation of this phenomenon, of particular importance in relation to the rate of spread of damage and the control of the beetle, the effect of the presence of decay in timber upon the duration of the life cycle of *Xestobium* has been the subject of extensive experimental work at Princes Risborough.

[1] The effect of *P. cryptarum* on the chemical composition of oak wood is discussed by Campbell & Bryant of this Laboratory. *Biochem. J.* 1940 (in the press).

Experimental

Material and methods

In view of the absence of published records of the rearing of *Xestobium rufovillosum* from egg to adult, either out-of-doors or in the laboratory, under known environmental conditions, the principal object of the preliminary experiments was to determine the condition of timber in which the insect could be bred and ascertain the duration of its life cycle under different conditions. The subject of the present paper, therefore, forms only part of a study of the biology and control of the death-watch beetle. When this work was begun, little was known of the possible significance of decay in timber in relation to infestation by *Xestobium*, but in the course of general life-history studies the importance of fungal decay came to light and confirmed the conclusions reached from field observations in trees and buildings. With the acquisition of this knowledge the enquiry has since been carried a step further by ascertaining from experiment the effect of different types and extent of decay upon the rate of development of the insect. The whole programme of work has extended over a period of years; the habits of the insect and its extremely slow rate of development under some of the conditions investigated have necessarily prolonged the experiments and delayed publication, pending the acquisition of as complete results as possible.

The beetles used were collected from willow trees during the emergence period in early spring (Fisher, 1938) or were obtained at the same time of year from infested timbers removed from buildings and stored under cover out-of-doors. They were confined for egg-laying on the wood in which the rate of larval development was to be determined either in vessels kept at different temperatures and in which the relative humidity of the atmosphere was controlled by mixtures of sulphuric acid and water (Wilson, 1921), or stored in a constant temperature-humidity room, or kept out-of-doors protected from sun and rain. By such means it was possible to determine the duration of the life cycle under conditions similar to those maintained in the investigation on the factors affecting oviposition (Fisher, 1938). Most of the work was carried out with English oak (*Quercus sessiliflora*) and English crack willow (*Salix fragilis*), but a few other timbers were also used. The general condition of the timber material with regard to the presence or absence of fungi before exposure to insect attack and other data on the samples used are included in the tables of results.

Results

It has previously been shown that, given a choice of decayed and sound wood each provided with cracks, crevices or roughened surfaces suitable for egg-laying, death-watch beetles do not show any preference for the decayed timber, nor is the viability of the eggs affected by the type of timber in which they are laid. Moreover, since the insect feeds only in the larval stage the behaviour of the larvae on emerging from the eggs and the duration of the larval stage as a whole can be taken, other things being equal, as a measure of the suitability of the wood for the development of the species. Compared with the length of larval life, the egg and pupal stages are short and have little effect on the duration of the life cycle under different environmental conditions. Temperature and humidity, on the other hand, have been shown to be important factors governing the rate of development of the insect, and it is therefore necessary to examine the effect of fungal decay by comparing the results of experiments carried out under similar conditions.

Duration of life cycle in sound and decayed willow (sapwood and heartwood not distinguished)

The earliest experiments upon the effect of the presence or absence of fungal decay in timber upon the rate of development of the insect were carried out with willow and the following is a brief summary of typical results obtained with this timber:

Experiment	Condition of samples	Minimum life cycle (to emergence)	Remarks
	(a) Laboratory temperature: relative humidity not constant but initially 80–90% (equivalent moisture content 18–20%)		
H. 30/3*b* H. 30/3*c*	Free from decay	Not determined	Progress of attack very slow: live larvae less than $\frac{1}{8}$ grown after $9\frac{1}{2}$ years
H. 30/6*a* H. 30/6*b*	Free from decay	—	Attack slight: died out after 6 years
H. 30/6*c*	Traces of decay in parts	$9\frac{1}{2}$–10 years*	Live larvae less than $\frac{1}{2}$ grown also present after same period
H. 32/3*c*	Decayed: fungus not identified: reduced to powder after 2 years, when additional wood decayed by *Coniophora cerebella* added	$3\frac{1}{2}$ years	Attack extensive
H. 32/3*d*	Slightly decayed by *Ganoderma applanatum* (Pers.) Pat.—white rot	$3\frac{1}{2}$–4 years*	—
H. 32/3*f*	Decayed but not uniformly by *Ganoderma applanatum*	Not determined	Attack progressed in decayed parts for 4 years
	(b) At 22–25° C.: relative humidity maintained at 80–90%		
(i) Decayed by *Coniophora cerebella* (brown rot).			
H. 33/4	Severely decayed	13 months	Attack extensive: samples rapidly powdered
X. 33/E.H. 1 2	Decayed in patches only	—	Attack confined to decayed patches; died out
X. 33/E.H. 3	Samples cut from same material and severely decayed throughout	12–17 months*	—
4		15 months	—
4A		11 months	—
X. 34 E.H. 2		16 months	Bred from beetles from willow
3		12–13 months	Bred from beetles from oak
X. 34/E.H. 8		10–11 months	2nd generation: bred under conditions of expt.
X. 34/B. 5		Less than 16 months	2nd generation: bred from beetles from expt. H. 33/4
X. 35/E.H. 5		15 months	2nd generation: bred from beetles from expts. X. 34/E.H. 2 and 3
(ii) Decayed by *Polystictus versicolor* (white rot).			
X. 34/E. 9	Severely decayed	—	Slight attack, died out
X. 36/1	Severely decayed	16–24 months*	—
	(c) Out-of-doors—protected from rain and sun, or in unheated hut		
X. 32/7	Slightly decayed in patches by *Ganoderma applanatum*	Not determined (cf. X./32/7—oak sapwood)	Attack slight: progressed very slowly: minute live larva present after $4\frac{1}{2}$ years
X. 36/2	Moderately decayed by *Ganoderma applanatum* or *Polystictus versicolor*	4 years	Emergence to date from *Polystictus versicolor* samples only: attack slight

* No examination of samples in the interval quoted.

In addition to the above, other records were obtained of the progress of attack in control samples of apparently sound willow exposed to infestation along with or in the absence of decayed wood. Although initial boring took place in the sound as well as decayed samples, the rate of progress was much more rapid in the latter; in the majority of experiments the attack died out in undecayed wood before the larvae had penetrated beneath the outer roughened surface. In a few cases they continued to live, tunnelling and growing very slowly. For instance, larvae 3 mm. in length, about one-third grown were found in such wood in which they had been living for $9\frac{1}{2}$ years. The presence of traces of fungal decay in parts of a sample (H. 30/6 *c*) may explain the completion of the life cycle of the insect by one individual after a larval stage prolonged for more than 9 years. In some circumstances, therefore, larvae of the death-watch beetle can continue to live slowly and even complete their development in willow apparently free from or with slight traces only of the presence of wood-destroying fungi.

In decayed wood the progress of attack is much more rapid and the duration of the life cycle markedly shorter. Moreover, willow severely decayed by fungi of the white or brown rot types appears to be more suitable for rapid larval development than sound or slightly decayed wood. In experiments at 22–25° C. and 80–90% relative humidity, attack sometimes died out in samples in which the decay was not uniform, after the decayed portions had been disintegrated. In willow severely decayed throughout, however, the insect completed its development in the very short period of 10–17 months. Such variation under similar conditions of temperature and humidity is probably due to differences in the distribution and severity of decay which may be related to variation in the relative amount of sapwood and heartwood present in individual samples.

It is noteworthy that in this work similar results were obtained whether the beetles used for egg-laying to start the experiment were obtained from oak or from willow. There was no indication of the unsuitability of a race of oak beetles for breeding in willow. When infestation of oak was attempted it was found that beetles collected from willow could be bred in that timber, and with similar results.

Furthermore, although rearing the death-watch beetle at temperatures above those normally occurring out-of-doors or in the roofs of buildings, slightly curtails the period spent by the adult in the wood under natural conditions prior to emergence, thereby shortening the duration of the complete life cycle, the results of these preliminary experiments with sound and decayed willow show that, irrespective of temperature, the presence or absence of decay has a marked effect on the progress of attack and the length of larval life of *Xestobium* in that timber. This effect has been observed with beetles of the second generation reared in decayed timber under the conditions of the experiment.

Duration of life cycle in sound and decayed oak

The majority of the experiments were carried out with the sapwood of English oak but the comparative suitability of heartwood was also determined. The results are summarized below and the conditions are given under which the different groups of experiments were undertaken. Except where otherwise stated, the samples used were cut from sound oak decayed in the laboratory by the fungi named, before exposure to insect attack.

Sapwood

Experiment no.	Minimum life cycle (to emergence)	Remarks
(*a*) Laboratory temperature: relative humidity not constant but initially 80–90% (equivalent moisture content 18–20%): decayed by *Phellinus cryptarum* (white rot)		
H. 32/3*a*	4 years	Decay not severe: insect attack quite extensive
H. 32/3*b*	—	Decay very slight: initial tunnels only, attack dying out early
X. 33/G. 2	3 years	Decay moderate: attack extensive
XF. 32/4	? 5 years (not emerged at 4½ years)	Decay slight. Laboratory conditions of relative humidity (cf. XF. 32/1)
(*b*) At 20–25° C.: relative humidity as above: decayed by *Phellinus cryptarum*		
H. 32/1*a* (20° C.)	2 years 6 months	Decay not severe: attack extensive: samples powdered.
H. 32/1*b* (20° C.)	Not determined but between 4½ and 7½ years	Control samples, not decayed
H. 33/3 (20° C.)	3 years 6 months	Decay moderate: attack severe
X. 32/7*c* (22° C.)	2 years 5 months	Exposed to attack along with decayed willow which did not become infested
X. 34/B. 4 (23° C.)	1½–2 years	From eggs laid by beetles from H. 32/1, giving adults of a 2nd generation bred under conditions of expt
H. 32/2*a* (25° C.)	2 years 2 months	Decay not severe
H. 32/2*b* (25° C.)	Not determined but not less than 6 years	Control samples, not decayed but moulds probably present
H. 33/2 (25° C.)	3–3½ years	Fresh mycelium on surface of samples: attack extensive
(*c*) At 22–25° C.: relative humidity maintained at 80–90%		
(i) Decayed by *Phellinus cryptarum.*		
X. 33/G. 5	14–15 months	Decay severe
X. 34/H. 1	18 months	Decay severe
X. 34/H. 4	23 months	Decay moderate
X. 34/H. 6	Not determined but more than 2 years	Decay slight: sound heartwood in sample
X. 35/2	17 months	Decay severe
X. 36/1	15–16 months	Decay severe
XL. 37/1*d*	16 months	Decay severe
(ii) Decayed by *Polystictus versicolor* (white rot).		
X. 35/H. 1 X. 35/H. 2	19 months	Decay severe: eggs by beetles bred in decayed willow (X. 33/E.H. 3–5)
X. 35/H. 3	18 months	Decay severe
(*d*) Out-of-doors—protected from rain and sun, or in unheated hut		
(i) Decayed by *Phellinus cryptarum.*		
XF. 32/1	7 years	Decay slight: attack not extensive
X. 32/7	5 years	Decay moderate: first record of duration of life cycle out-of-doors. Maximum emergence after 6 years (cf. X. 32/7—willow)
X. 35/1	3 years	Decay severe: maximum emergence after 4 years
X. 36/2	3 years	Decay severe: emergence after 4 years, from decayed willow exposed to attack along with oak
XF. 31/1 XF. 31/5 XF. 31/6	—	Decay very slight: attack died out early in two samples but in two others live larvae ⅓–½ grown after 8½ years
XF. 31/C	—	Control to above: decay absent: similar live larvae after 8½ years
(ii) Decayed by other fungi.		
X. 33/G. 4	4 years	Decayed to moderate extent by *Polystictus versicolor*: *Stereum hirsutum* or *Merulius lacrymans.* Maximum emergence after 5 years
X. 32/6	6 years	Sections of decayed branch-wood collected in the field: fungi not identified, but probably *Polystictus versicolor*
XF. 32/5*a–c*	—	Superficial growth of mould (*Paeciliomyces varioti*): no attempt at larval tunnelling: free from attack

Although the variation in the length of the life cycle in the above different groups is in particular due to temperature and moisture content of the wood, the distinct variations within each group can best be explained by differences in the extent of decay in the various samples. For instance, under laboratory conditions of temperature and an initially high but falling moisture content of only slightly decayed wood, the life cycle occupied from 3 to 5 years. On the other hand, at 22–25° C. and a constant moisture content of 18–20%, the insect completed its development in severely decayed wood in as short a period as 14–15 months as compared with a minimum period of 11 months in severely decayed willow. Where the decay was slight, however, the life cycle was prolonged.

The effect of the presence of decay and an indication that an increase in its severity tends to shorten the life cycle is well illustrated by the results of the experiments out-of-doors. In severely decayed wood in a condition similar to the natural habitat of the insect in trees, the life cycle can be as short as 3 years, but may be prolonged to 7 years or more when the decay is less. In general, therefore, oak sapwood decayed by wood-destroying fungi, irrespective of species, is much more suitable for rapid larval development than sound sapwood. The presence of a superficial growth of mould fungi does not have the beneficial effect of the wood-destroying species for preparing the way for infestation by the insect. Instances were recorded (H. 32/1 *b* and 2 *b*) of the completion of the life cycle in apparently sound sapwood after a prolonged larval period during which the amount of tunnelling was slight in comparison with that by larvae completing their development in a shorter time in decayed wood. As in willow, however, other instances (H. 32/3 *b*) were noted in which first stage larvae, attempting to establish themselves in sound oak sapwood, succeeded only in tunnelling superficially amongst the raised chips of roughened surfaces before dying within a few weeks.

Heartwood

The comparative suitability of recently seasoned and old oak heartwood, sound and decayed, for infestation by *Xestobium* is shown in the following table of results of infestation experiments:

Experiment no.	Condition of heartwood	Minimum life cycle (to emergence)	Remarks
	At 22–25° C.: relative humidity 80–90%		
XL. 37/1 *a*	Old oak from buildings: wood-destroying fungi absent. *Penicillium* sp. present	—	Attack progressing slowly: live larvae ⅓–½ grown after 3 years
XL. 37/1 *b*	New oak with no trace of fungi present	—	Initial tunnels only in fibres on rough surfaces: attack died out early
XL. 37/1 *c*	New oak decayed by *Phellinus cryptarum*	16 months	Decay severe and attack extensive
H. 33/1 *a*	Oak heartwood showing typical brown colour due to early stage of decay by *Fistulina hepatica*	—	Tunnels and small live larvae after 7 years
H. 33/1 *b*	Heart- and sapwood free from wood-destroying fungi: moulds probably present	—	Controls to (*a*). Heartwood—initial attack only, died out early. Sapwood—live larvae. ½–¾ grown, but life cycle not completed, after 7 years

Whilst no attack took place in sound new heartwood, when it had been severely decayed by *Phellinus cryptarum*, it was as suitable for infestation and larval development as decayed sapwood, the duration of the life cycle in both being as short as 16 months (XL. 37/1 *c*, *d*), under similar conditions of temperature and humidity. The apparent preference of the insect for sapwood is therefore due to its greater liability to fungal decay, rendering it susceptible to infestation before the more durable heartwood.

Attack took place in old oak heartwood free from wood-destroying fungi but the rate of larval progress and growth was slow. On the other hand, although samples of brown oak heartwood became infested, the development of the larvae was no more rapid than in normal sound heartwood and slower than in sound sapwood.

Duration of life cycle in other woods

In view of the apparent relation between fungal decay and suitability for *Xestobium* attack in the timbers normally infested by this insect, additional experiments with other woods were undertaken with the following results:

Experiment no.	Condition of wood	Minimum life cycle	Remarks
	At 22–25° C.: relative humidity 80–90%		
XL. 37/1 *d*	Banak (*Virola merendonis*), severely decayed, naturally: fungus not identified	12 months	Attack extensive: wood severely powdered
XL. 37/1 *e*	Scots pine (*Pinus sylvestris*), decayed by *Coniophora cerebella*	—	Decay very severe. Numerous tunnels but only 1 live larva, ½–¾ grown, after 2½ years
X. 38/1	Sitka spruce (*Picea sitchensis*), decayed by *Phellinus cryptarum*	—	Decay severe: small tunnels quite abundant but only 2 small larvae alive after 1½ years
X. 38/5	Balsa (*Ochroma* sp.), sound, free from decay	—	Initial attack only: died out early. Numerous small tunnels but nothing alive after 1½ years

The rapid development of the insect in banak, a non-durable hardwood from British Honduras, of which supplies were available at the Laboratory at the time of this work, is comparable to the results obtained with decayed willow and confirms the conclusions reached from the earlier experiments that hardwoods, provided they are severely decayed, are particularly suitable for death-watch beetle attack. Coniferous timbers, on the other hand, although severely decayed, are much less suitable and although liable to infestation, the rate of growth of the larvae and progress of attack are markedly slower than in hardwoods, even when decayed by the same fungi (X. 38/1). Balsa was included in this set of experiments to determine the rate of development of the insect in a sound timber of very low density which might be expected to offer low resistance to larval boring as compared with oak and other timbers of much higher density. The apparent unsuitability of the wood when undecayed is, therefore, worthy of special note. No experiments were undertaken with decayed balsa.

Discussion and conclusions

All the evidence obtained from field observations and laboratory experiments clearly proves that the presence of decay in timber has an important beneficial effect upon its suitability for *Xestobium* larval development, governing the length of life cycle of the insect. Temperature and moisture content are also controlling factors, but even when these are

suitable the condition which finally determines the degree of suitability of wood for rapid death-watch beetle attack is the presence of decay. There does not appear to exist any relationship between the insect and a specific fungus and, furthermore, *Xestobium* has been recorded from and bred in timber decayed by fungi of both the white and brown rot types. The results of the present work, however, do not show whether the one type of decay renders timber more suitable for the development of the insect than the other. There are indications that the rate of larval growth in decayed wood is more rapid in hardwoods of low density, e.g. willow and banak, than in heavier timbers, e.g. oak. It has yet to be shown, however, whether this is the result of fungal decay being more rapid and having a more marked effect on the physical condition (e.g. hardness) of timbers of low density or to concurrent chemical changes affecting the nutritional value of wood for the growth of the larvae. In this connexion, however, it is noteworthy that although it was not possible to rear the insect in sound balsa is was bred, albeit after a prolonged larval period, in sound oak and willow. Consequently, although the results of the experiments show that the extent of decay is apparently of prime importance, further work with woods of both high and low densities, decayed to varying degrees by fungi producing different types of rot, is required before the true significance of this insect-fungus relationship can be determined satisfactorily. So far as the effect of fungal decay on the nutritional value of the wood to the larva is concerned, this problem has been dealt with in co-operation with the Wood Chemistry Section, whose results are about to be published.

In the meantime, it is of interest to examine the results of the present work in relation to the occurrence of *Xestobium rufovillosum* in buildings. The comparative unsuitability of sound timber for death-watch beetle attack is related to the difficulty with which first stage larvae on emergence from the egg can succeed in boring into hard surfaces. It was frequently observed that although such larvae were able to tunnel in the raised fibres of roughened surfaces of sound wood, they did not often succeed in penetrating the hard timber beneath. In the few cases in which they established themselves, tunnelling and development continued slowly and after a prolonged larval period the insect sometimes completed its development. It would seem, therefore, that when first stage larvae, wandering as is their habit on emergence, attempt to bore in undecayed or slightly decayed timber, the mortality is high and the rate of development of the few individuals which may succeed in becoming established, slow with a corresponding small amount of tunnelling. In contrast to this, first stage larvae can succeed in boring into decayed wood, their subsequent rate of tunnelling and development depending upon the extent of decay present. In a building where the structural timbers are of oak or other hardwood, and conditions are favourable for fungal infection, as is of frequent occurrence under faulty roof coverings or near defective rain pipes, behind panelling or in unventilated flooring, rapid spread of death-watch beetle attack is likely with resultant extensive damage. In this connexion, the common practice in the past of using rough-hewn green oak of large dimensions for the heavy roofing timbers in buildings has no doubt involved the use of trees in which decay, active or incipient, was present, with the result that the timber, if not infested by the insect at the time of insertion in a building, was in a condition suitable for attack subsequently, and would explain the widespread occurrence of death-watch beetle damage, often no longer active at the present day, in such old buildings.

Where decayed softwoods occur, as in flooring, conditions are also favourable for spread

of attack. In view, however, of the comparative unsuitability of coniferous timbers for larval development, it is probable that damage to such woodwork is less the result of direct infestation by egg-laying beetles than by partly grown larvae making their way into softwoods from severely infested hardwoods with which they are in contact.

The presence of decayed timber in a building is a dangerous focus of infestation and re-infestation by the death-watch beetle but the results of the experiments and of observations in attacked buildings show that, once infestation has taken place, larvae boring at first in fungus-infested wood can pass in the course of their tunnelling to adjacent less decayed or sound timber. Although the rate of development and the extent of damage are thereby decreased, these larvae can continue to live and complete their development. Such is an explanation of the extensive death-watch beetle damage commonly associated with oak structural timbers in old buildings in which sapwood and heartwood apparently sound, as well as decayed, have become seriously damaged. The rate of progress of infestation and its frequent cessation is governed not only by the extent and severity of decay in the timbers but also by moisture conditions, and by temperature.

In addition to the timber species concerned, the three factors, extent of decay, moisture and temperature, together determine the duration of the life cycle of the death-watch beetle, principally by their effect on the length of the larval stage. The results of the present studies suggest that under the conditions of temperature and humidity which normally occur in old buildings such as churches, the minimum life cycle in badly decayed oak is two years. In the majority of such buildings, however, where the timbers are not extensively decayed, it is considerably longer and may be extended by several years. Although no direct observations have been made on the length of the life cycle of the insect in decayed parts of trees, the results of experimental work out-of-doors with severely decayed timber suggest that the minimum life cycle of the insect in its natural habitat is three years.

Summary

The present paper, the third in a series on the biology of the death-watch beetle, *Xestobium rufovillosum* De G., describes investigations on the effect of decay upon the suitability of timber for infestation by this insect.

A list is given of the fungi found in the natural habitat of the beetle in the decayed parts of oak and willow trees.

The occurrence of damage in structural timbers in buildings is discussed briefly, and it is shown that most severe infestation is usually found in decayed wood. Eight species of fungi, mostly wood-destroying species causing both white and brown rots, have been identified from insect-attacked timbers.

The effect of the presence or absence of fungi on the rate of larval development and on the duration of the life cycle in oak, willow and a number of other timbers has been determined, under different conditions of temperature and humidity. The length of the life cycle ranges from one to ten years or more, according to environmental conditions.

It is shown that timber attacked by wood-destroying fungi is more suitable for the development of the insect than sound or mould-infested timber, and there are indications that an increase in the extent of decay tends to decrease the duration of the life cycle.

It is concluded that although temperature and humidity have an important effect on the

rate of development of the death-watch beetle, the presence of decay finally determines the degree of suitability of timber for infestation.

The bearing of this fungus-insect relationship upon the spread of infestation in buildings is discussed.

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REFERENCES

Braid, K. W. (1924). Some observations on *Fistulina hepatica*, and hollow stagheaded oaks. *Trans. Brit. mycol. Soc.* **9**, 210.

Cartwright, K. St G. & Findlay, W. P. K. (1936). *The Principal Rots of English Oak*. London: H.M.S.O.

Fisher, R. C. (1937). Recent work on wood-destroying insects. *J. R. Soc. Arts*, **85**, 407–25.

—— (1938). Studies of the biology of the death-watch beetle, *Xestobium rufovillosum* De G. II. The habits of the adult with special reference to the factors affecting oviposition. *Ann. appl. Biol.* **25**, 155–80.

Lefroy, H. M. (1924). The treatment of the death-watch beetle in timber roofs. *J. R. Soc. Arts*, **72**, 260–6.

Mangin, L. & Patouillard, N. (1922). Sur la destruction des charpentes au chateau de Versailles par le *Phellinus cryptarum* Karst. *C.R. Acad. Sci., Paris*, **175**, 389.

Ramsbottom, J. (1937). Dry rot in ships. *Essex Nat.* **25**, 231–67.

Williamson, H. S. (1923). The origin of "golden oak". *Ann. Bot., Lond.*, **37**, 433.

Wilson, R. E. (1921). Humidity control by means of sulphuric acid solutions with critical compilation of vapour pressure data. *Industr. Engng Chem.* **13**, 326–31.

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AEROBIC DENITRIFICATION

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(With 8 Text-figures)

Denitrification is the reduction of nitrates to gaseous nitrogen, sometimes mixed with oxides of nitrogen: it is the only process which certainly causes a loss of nitrogen in nature, because the nitrogen gas escapes into the atmosphere. It is known that nitrite certainly, and probably hyponitrite, are formed as intermediates in the course of the reaction; it is obvious that nitrate (or nitrite) must be present if denitrification is to take place; and it is also known that a compound to act as a hydrogen donator is necessary for the process. Karlsen (1938) published a detailed study of the effect of *p*H and of iron on denitrification by *Pseudomonas aeruginosa*; and Elema (1932) and Elema *et al.* (1934) studied the changes in oxidation-reduction potential during denitrification by *Micrococcus denitrificans*. Elema's paper includes a set of equations which represents the most probable course of the reaction.

Several details of the process remain obscure, among them being the effect of aeration. The generally accepted view is that denitrification is an essentially anaerobic process, and that it is diminished or stopped by the admission of air. This supposition is probably accepted mainly because it provides a neat teleological explanation for the fact of denitrification; oxygen is made available by the reduction of nitrate; so under anaerobic conditions the bacteria reduce the nitrate in order to supply themselves with oxygen. Under aerobic conditions they need not reduce nitrate to obtain oxygen, and it is therefore assumed that they do not reduce it. This supposition is based on very little experimental evidence; though Weissenberg (1902) described a strain of *Pseudomonas aeruginosa*, which grew on nitrite under aerobic conditions, but did not reduce it, while in anaerobic cultures the nitrite was all reduced with much gas production. There is no reason to suppose, however, that every denitrifying species is affected by aeration in the same way. Seiser & Walz (1925) found that nitrogen was lost from both aerobic and anaerobic cultures of *Ps. putida*. The effect of aeration on *Ps. denitrofluorescens* was studied by Korochkina (1936), who remarks "a relatively high *r*H value does not exclude denitrification. Therefore it is very difficult to eliminate denitrification by means of increased aeration."

The experiments described in the present paper were mainly concerned with the effect of aeration on denitrification; they were performed with pure cultures of two denitrifying species in simple synthetic liquid media.

Source and description of bacteria

The two species of denitrifying bacteria used in this work are classifiable under the genus *Pseudomonas*, as defined by Dowson (1939). It is not possible to identify either of them with any previously described species, especially as they produce fluorescent pigment in a spasmodic and unpredictable manner only. They are, therefore, referred to as *Pseudomonas* sp., strain N 8, and *Pseudomonas* sp., strain 309.

Pseudomonas sp., strain N 8. Isolated from Harpenden soil by incubating a small quantity of soil in Giltay's medium, and plating on Giltay agar. Cultural characteristics: rods, about $1 \cdot 0 \times 0 \cdot 5 \mu$, motile with 1–2 polar flagella, non-spore forming, Gram-negative; agar streak: growth good, colourless, almost transparent, smooth, edge undulate, occasionally produces green fluorescent colour in agar, and also in liquid media. Liquefies gelatine rapidly. Produces acid but not gas on glucose, fructose and sucrose; lactose is not fermented. Reduces nitrates to nitrates and gaseous nitrogen. Slightly peptonizes litmus milk without change in acidity. Aerobic.

Pseudomonas sp., strain 309. Isolated from a small-scale septic tank in which milk washings were being treated in the laboratory. Cultural characteristics: rods, $1 \cdot 0 \times 0 \cdot 7 \mu$, motile with 1 polar flagellum, non-spore forming, Gram-negative; agar streak: growth good, colourless, translucent, faintly granular, edge undulate, occasionally produces green fluorescent colour in agar and in liquid media. Liquefies gelatine rapidly. Produces acid but not gas on glucose, fructose, galactose, mannitol and glycerol; lactose, sucrose and maltose are not fermented. Reduces nitrates to nitrites and gaseous nitrogen. Slightly peptonizes litmus milk without change in acidity. Can decompose fat. Aerobic.

Methods

Media. The synthetic liquid media employed were made up in the following neutral basis solution (Klaeser, 1914):

KH_2PO_4	0·5 g.	in 1 l. distilled water.
K_2HPO_4	0·5 g.	
$CaCl_2$	0·1 g.	
NaCl	0·1 g.	
$MgSO_4, 7H_2O$	0·3 g.	
$FeCl_3$	Trace	

For alkaline media the mixture of phosphates was replaced by 1 g. K_2HPO_4. The medium most commonly used also contained:

KNO_3	1·0 g. (139 mg. nitrogen) per litre.
Glucose	3·5 g. (1400 mg. carbon) per litre.

In some cases the nitrate was wholly or partly replaced by nitrite, to give about 140 mg. nitrogen/l., or increased in amount; but no other sources of nitrogen were used. Glucose was replaced in different experiments by a variety of organic compounds, all in sufficient quantity to give 1400 mg. carbon/l., and so a carbon/nitrogen ratio of 10. Organic acids were used as the sodium salt; and ethyl alcohol, when used, was added after steaming. The basis solution and nitrate were autoclaved, the glucose added and the *p*H adjusted to 7·0 or 8·0; the complete medium was measured out into the flasks to be used in the experiment, and steamed for 1 hr.

Inoculation and incubation

In two experiments the medium was contained in test tubes with Durham tubes, which were inoculated directly from young agar slope cultures. In all other experiments the medium was contained in conical flasks, and was inoculated as follows (Meiklejohn, 1937): a heavy suspension of the growth from a young agar slope was made in 10–25 ml. of sterile basis solution, and the number of cells counted. The volume of this suspension required to give an initial count of about 10 million bacteria/ml. was then pipetted into each flask. All flasks were incubated at room temperature and in the light, and were maintained at three levels of air supply, referred to as aerated, control and anaerobic conditions respectively. *Control* flasks were plugged with cotton-wool and were undisturbed except for sampling. They contained a fairly shallow layer of medium, not exceeding 2 cm. in depth. *Aerated* flasks were of the same size, and contained the same volume of medium, as the controls. The cotton-wool was replaced by a sterilized rubber stopper carrying inlet and outlet air tubes, and a small steady stream of air, filtered through cotton-wool, was bubbled through the medium. *Anaerobic* flasks were smaller and therefore contained a deeper layer of medium. They were stoppered with cotton-wool, and were incubated in a closed vacuum desiccator containing alkaline pyrogallol, under reduced pressure. Samples were taken from all flasks at the beginning and end of incubation, and usually during its course.

Examination of samples

Number of cells. The bacteria were counted throughout by the direct method with a Thoma haemocytometer. Duplicate counts were always made. This method of counting includes all bacterial cells present, whether viable or not. *p*H *determinations* were made colorimetrically with a Hellige comparator. *Nitrite* was detected by the Griess-Ilosvay reagent, and estimated by comparing a 1 ml. sample against a set of dilutions of a standard solution of sodium nitrite. If the nitrite test was negative, *nitrate remaining* in the medium was tested for by adding zinc dust to the nitrite sample and reagent, as suggested by ZoBell (1932).

The nitrogen estimation methods were primarily chosen for use with very small samples, as it was desired to alter the volume of medium as little as possible during the course of an experiment. All analyses were done in duplicate. *Total nitrogen* was determined, on 1 ml. samples, by a modified Kjeldahl method. If nitrate was present in the samples, it was first reduced with iron in acid solution, after the method of Pucher *et al.* (1930). The sample was then digested with concentrated sulphuric acid and a little potassium sulphate and copper sulphate; and the ammonia formed was estimated by Woolf's method (1928), in which the ammonia is expelled by aeration in presence of excess of alkali, caught in boric acid solution containing brom-cresol green, and titrated directly with sulphuric acid made up in the same boric acid-indicator solution. The strength of the acid used for titration was about $N/200$ (1 ml. = 0·0715 mg. N). *Non-protein nitrogen* was determined by the same method, after precipitation with basic lead acetate (Stiles *et al.* 1926). Blank determinations were made on the complete reagents used in each method. The blank values obtained were: *Kjeldahl*: 0·011 mg. N/ml. *Reduction* followed by Kjeldahl: 0·025 mg. N/ml. *Protein precipitation* and Kjeldahl: 0·015 mg. N/ml. These blanks have been subtracted from all the nitrogen values given in the tables and text-figures.

Results

A. *Effects of various organic compounds*

Both species grew abundantly on the simple synthetic media used, with nitrate as the only source of nitrogen, if a suitable organic compound was supplied as a substrate. Several organic compounds were found to act as substrates for growth; but not all would act as hydrogen donators for the denitrification reaction. The only certain proof of denitrification is actual measurable loss of nitrogen; but as nitrogen gas is the end product, and nitrite an intermediate product, of denitrification, the appearance of gas in a culture, or the appearance and subsequent disappearance of nitrite, are indications that the reaction has taken place. The appearance of gas was regarded as an indication of denitrification in the following test-tube experiment; both species were grown on several different sugars, and on sodium lactate, under the following conditions: air supply *control*, *p*H 7·0, KNO_3 0·1 %, C/N ratio 10.

Table 1

		Glucose		Sucrose		Galactose		Lactose		Lactate	
Species	Days	NO_2	Gas	NO_2	Gas	NO_2	Gas	NO_2	Gas	NO_2	Gas
309	3	0	+	6	0	60	0	6	0	60	+
	7	0	+	1	0	0	+	7	0	0	+
N 8	3	0	+	0	+	.	.	3	0	2	+
	7	0	+	0	+	.	.	15	0	0	+

NO_2 as mg. N/l.

Visible growth was seen in all the tubes, and from the appearance of gas it is evident that both species denitrify on glucose and lactate, N 8 on sucrose and 309 on galactose. It is also evident that neither strain will denitrify on sugars it does not ferment; 309 does not ferment sucrose, and only produces a little nitrite, and no gas, on sucrose. Neither strain ferments lactose, and does not denitrify on it.

Figs. 1 and 2 give the results of another set of experiments, in which species 309 was grown on six different organic compounds under these conditions: air supply *control*, *p*H 7·0, KNO_3 0·1 %, C/N ratio 10. In this case the formation and disappearance of nitrite was taken as an indication of denitrification; as no nitrate was left in the medium after the nitrite had disappeared, it is fairly safe to assume that denitrification really did take place.

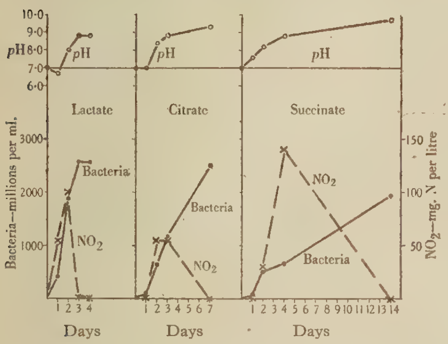

Fig. 1.

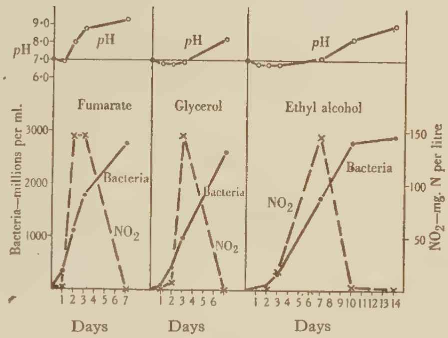

Fig. 2.

Fig. 1. Growth of species 309 and apparent denitrification on lactate, citrate and succinate.
Fig. 2. Growth of species 309 and apparent denitrification on fumarate, glycerol and ethyl alcohol.

The nitrite curves in Fig. 1 show that the denitrification reaction has taken place in all three cases. Species 309 can apparently use lactate, citrate, and succinate not only as substrates for growth, but also as hydrogen-donators in the reduction of nitrate. In each case the *p*H values show an alkaline drift, though the final *p*H was not the same. Species N 8 was also grown on lactate with almost identical results.

Three more compounds, sodium fumarate, glycerol and ethyl alcohol, can be used as hydrogen donators for the reduction of nitrate by species 309. On alcohol, growth was a little delayed, but high numbers of bacteria were finally produced on all three compounds; there was an alkaline drift in *p*H in the later stages after an initial slight acidity. Figs. 1 and 2 therefore include six cases in which nitrate was apparently completely reduced under aerobic conditions; the reduction was accompanied by good growth of the bacteria and an alkaline *p*H drift.

Fig. 3 gives three cases in which complete denitrification did not take place. Two of the experiments illustrated were performed under control air supply, and the third in anaerobic conditions; other conditions were: *p*H 7·0, KNO_3 0·1 %, C/N ratio 10.

The first case was a culture of 309 on glucose; when the experiment recorded in Table 1 was performed, 309 could denitrify in presence of glucose, but shortly afterwards it lost that power. When it was grown on glucose the medium became very acid, and nitrite accumulated and did not disappear. The total bacterial count was 800 millions/ml. at the end of the experiment, but it probably included very few viable cells, as attempts to subculture from this flask were unsuccessful. Another attempt was made to grow 309 on glucose, and in this case a *p*H value of 4·1 was reached in the culture after 4 days' incubation. The culture was then titrated back to *p*H 6·2 with weak alkali, but no further growth or reduction of the accumulated nitrite resulted. Glucose cultures of species N 8, on the other hand,

showed normal growth and complete reduction of the nitrate; a slight shift of *p*H to the acid side was always observed in the early stages; but no value more acid than 6·2 was ever observed. In the second case, a culture of 309 on malate, the cause of the failure of denitrification must be different, for the bacteria grew well, and a very alkaline *p*H value was attained. The third case of failure to denitrify is an anaerobic culture of species N 8 on ethyl alcohol; under the same conditions 309 grows and denitrifies normally; but N 8 hardly grows at all, and merely reduces all the nitrate to nitrite and no further. In all three cases nitrite accumulates in the medium; apparently the first stage of denitrification—the

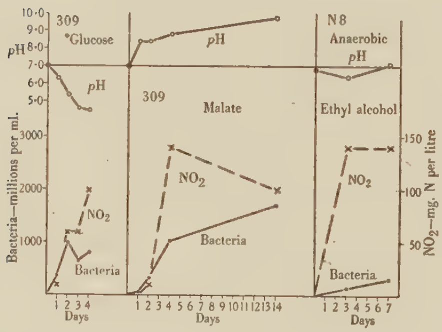

Fig. 3.

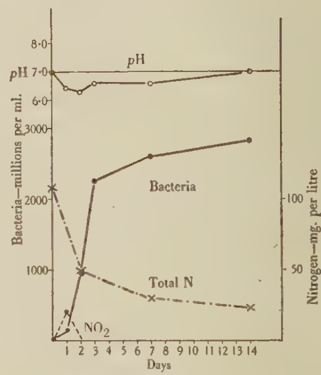

Fig. 4.

Fig. 3. Three cases of failure of denitrification.
Fig. 4. Course of events in a glucose-nitrate culture of species N 8 under control air supply.

formation of nitrite—is less sensitive to external conditions than the subsequent stages. A case where even the first stage of reduction did not take place was observed in a triple experiment in which an attempt was made to grow species N 8 on sodium acetate (0·793 %, KNO_3 0·1 %, C/N ratio 10, *p*H 7·0). One week after inoculation the numbers of bacteria, from an initial count of 9 million/ml., had only risen to 10 million in the aerated, 12 million in the control and 31 million in the anaerobic culture; the only change in *p*H was from 7·0 to 6·9 in the anaerobic flask, and no nitrite at all was found, but much nitrate was still present.

Not only the kind but also the quantity of substrate influences the course of denitrification, as is shown in Table 2, which gives the results of an experiment on wide variations in the C/N ratio, performed under the following conditions: air-supply *control*, *p*H 7·0, C-source sodium lactate, N-source KNO_3. The medium with C/N ratio 10 contained 0·1 % KNO_3 and 0·35 % lactic acid; these concentrations were varied to give the different ratios shown in Table 2.

Small quantities of nitrate are very rapidly reduced if the carbon source is present in sufficient quantity; but a deficiency in carbon source diminishes the growth of the bacteria, and at a C/N ratio of unity denitrification is not complete, and nitrite is left in the culture.

Table 2. 309 *at different* C/N *ratios*

Medium contains:

C mg./l.	N mg./l.	C/N	Days	NO_2 mg. N/l.	Gas	Growth
1400	139	10	1	60	o	Good
			7	o	+	
700	139	5	1	96	o	Fair
			7	9	+	
140	139	1	1	48	o	Poor
			7	48	+	
1400	70	20	1	70	o	Good
			3	o	+	
1400	28	50	1	25	o	Good
			3	o	+	

B. *Effect of initial* pH

To find the limits of pH value outside which denitrification could not take place, species 309 was grown in test-tubes on media made up at various initial pH levels; the mixture of phosphates in the basis solution was altered to give solutions buffered at the desired level. The other conditions of the experiment were: air-supply *control*, KNO_3 0·1 %, C-source sodium lactate, C/N 10.

Table 3. *Effect of initial* pH *on species* 309

Initial pH	Days	Final pH	Growth	NO_2 mg. N/l.	Gas	Nitrate left
3·9	3	$<$4·0	Just visible	o	o	+
	7	$<$4·0	Just visible	o	o	+
4·8	3	7·0	Good	100	o	.
	7	7·4	Good	o	+	o
5·4	3	6·6	Good	100	o	.
	7	7·4	Good	o	+	o
7·0	3	8·2	Good	o	+	o
	7	8·4	Good	o	+	o
9·5	3	$>$9·0	Just visible	o	o	+
	28	$>$9·0	Poor	60	o	.

To judge by the appearance of gas, denitrification was complete in 3 days at initial pH 7·0. At initial pH 4·8 and 5·4 it was delayed, but at the end of 3 days the pH had shifted far enough to the alkaline side for denitrification to proceed normally. At the extreme pH values of 3·9 and 9·5 denitrification did not take place. In later experiments denitrification took place normally in media at initial pH 8·0 (cf. Karlsen, 1938).

C. *Effect of air supply*

The question of the existence of denitrification under aerobic conditions is of interest; examples have already been given in Figs. 1 and 2 of apparent denitrification under control (undisturbed aerobic) conditions, but as no total nitrogen estimations were performed, it is not certain that there was an actual loss of nitrogen in these experiments. A fresh series of experiments was set up to investigate the effect of air supply in more detail; in this series conditions other than air supply were kept as constant as possible. The same species—N 8—was used throughout, and the medium had the same composition: glucose 0·35 %, KNO_3 0·1 %, C/N ratio 10, initial pH 7·0.

The results of a few examples from this series of experiments are given below. Determinations of total nitrogen were performed, so that any loss of nitrogen could be measured.

It was therefore possible to show with certainty whether denitrification did or did not take place. In Fig. 4 are given the data from a glucose-nitrate culture of species N 8 incubated under control conditions of air supply.

The curve of total nitrogen shows that there was a real loss of nitrogen, amounting to more than three-quarters of that originally present, from this culture grown as a shallow layer of liquid in an undisturbed aerobic flask. The rate of nitrogen loss was greatest in the first few days of incubation. The loss of nitrogen was accompanied by those changes in the culture that have already been indicated as signs of denitrification: (1) the bacteria grew well, (2) there was an early shift of *p*H to the acid side (characteristic of glucose cultures) followed by an alkaline drift, (3) nitrite was formed on the first day and then disappeared, (4) there was no nitrate left at the end of incubation, (5) on the second and third days of incubation, the culture had a "soda-water" appearance, caused by small bubbles of gas. Fig. 5 illustrates an experiment in which a control culture was compared with an aerated culture (through which air was bubbled).

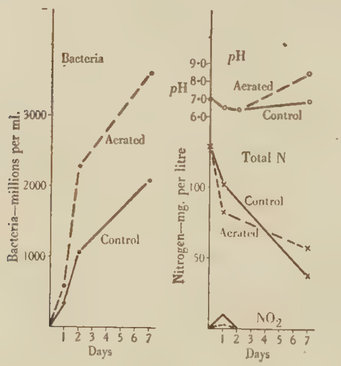

Fig. 5.

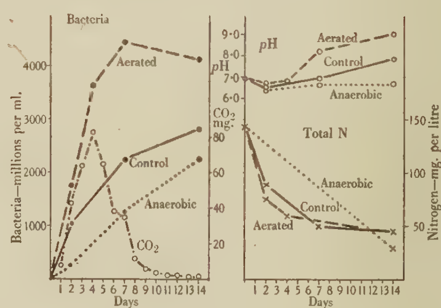

Fig. 6.

Fig. 5. Comparison of an aerated with a parallel control culture of species N 8.
Fig. 6. Comparison of three parallel glucose-nitrate cultures of species N 8: aerated, control and anaerobic.

In the first place it is evident that there was a considerable loss of nitrogen from both cultures, but slightly more nitrogen was left in the aerated culture at the end of the experiment than in the control. The numbers of bacteria were much higher in the aerated culture than in the control, and the final *p*H was more alkaline. These two effects of increased air-supply are also shown in Fig. 6, which gives the results of a three-fold experiment on aerated, control and anaerobic cultures.

More than two-thirds of the total nitrogen originally present was lost from all three cultures; least nitrogen was left in the anaerobic culture at the end of incubation. The numbers of bacteria were highest in the aerated culture, and lowest in the anaerobic, throughout the experiment; in the first few days of incubation there was an easily visible difference in turbidity between the aerated and anaerobic flasks. As in the last experiment, the growth of the bacteria appears to be stimulated by increased air-supply. The final *p*H value was progressively more alkaline as the air-supply was increased; subsequent experiments suggest that the difference in *p*H was probably due to the difference in amount of glucose used aerobically and anaerobically at a C/N ratio of 10. The carbon dioxide output of the aerated

culture was estimated by the Pettenkoffer method. In the 14 days of the experiment 371 mg. CO_2 were given off. A curve showing the rate of output of CO_2/24 hr. is included in Fig. 6; there is a sharp peak in the curve on the 4th day, towards the end of the logarithmic period of growth. Nitrogen was also lost most rapidly from the aerated culture in the earlier days of incubation. From the CO_2 curve it is evident that metabolic activity in the culture practically ceased after 10 days; it may therefore be assumed that the nitrogen estimation on the 14th day is a real measurement of the nitrogen left when the reactions accompanying growth were over.

Two examples have now been given where the numbers of bacteria were apparently increased by increased aeration; and this difference in numbers appeared throughout this series of experiments. To examine the reality of the difference, all the figures available were collected and compared. The difference between the bacterial counts taken on the same day from parallel cultures was set down; and the mean value of all the differences between control and anaerobic, aerated and control, and aerated and anaerobic parallels, was calculated, and an estimate made of the standard error of each of the three means.

Table 4. *Comparison of bacterial numbers at three aeration levels (all numbers in millions/ml.)*

Exp.	Age in days	Anaerobic cultures	Difference control-anaerobic	Control cultures	Difference aerated-control	Aerated cultures	Difference aerated-anaerobic
7	1	—	—	336	+ 242	578	—
	2	—	—	1058	+1230	2288	—
	7	—	—	2072	+1528	3600	—
8	1	—	—	242	+ 90	332	—
	2	—	—	1014	+ 508	1522	—
	7	—	—	2925	+1175	4100	—
9	2	—	—	648	+ 592	1240	—
	3	576	+ 772	1348	+2342	3700	+3124
	16	1410	+2140	3550	+ 850	4400	+2990
12	2	248	+ 800	1048	+ 700	1748	+1500
	7	1260	+ 980	2240	+2210	4450	+3190
	14	2240	+ 585	2825	+1300	4125	+1885
23 A	14	1490	+1070	2560	—	—	—
B	14	1520	+3380	4900	—	—	—
Number of cases			7		12		5
Mean value of difference			1400		1064		2538
Standard error of mean			±383·3		±205·8		±351·9
t (Fisher, 1936)			3·65		5·17		7·21

The values of t in each column are high enough to be significant for that number of cases. That is to say, each mean value is sufficiently large, when compared with its own standard error, to leave no doubt of its real existence. It can therefore be regarded as certain that, in this series of experiments, the numbers of bacteria are higher in control than in anaerobic cultures, and higher in aerated cultures than in either of the two other classes.

As well as the difference in bacterial numbers, a difference in the amount of nitrogen left in the cultures at the end of the experiment appears in Figs. 5 and 6; and in several other experiments it was observed that most nitrogen was left in aerated, and least in anaerobic, cultures. At the end of these experiments, a determination of the nitrogen left after precipitation with basic lead acetate—"non-protein nitrogen"—was made. By subtracting this figure, usually small, from that for total nitrogen, an estimate can be made of "protein"

nitrogen, which is presumably the nitrogen locked up in the cells of the bacteria. The following mean values for "protein" nitrogen were obtained from cultures with a C/N ratio of 10: aerated cultures (4 cases) 43 mg. N/l.: control cultures (7 cases) 31 mg. N/l.: anaerobic cultures (3 cases) 22 mg. N/l. There are not many figures available, and too high a value should not be placed on the accuracy of the "protein" nitrogen estimations, as they represent the difference of two determinations on small samples. All the available figures for "protein" nitrogen, and the numbers of bacteria present at the same time, are given in Table 5.

Table 5. *Bacterial numbers and precipitable nitrogen*

Experiment	Flask	Number of bacteria 100 millions/ml.	"Protein" nitrogen, mg./l.
5	Control	28·25	20
7	Control	20·72	30
	Aerated	36·00	57
8	Control	29·25	27
	Aerated	41·00	39
9	Anaerobic	14·10	14
	Control	35·50	29
	Aerated	44·00	53
12	Anaerobic	22·40	18
	Control	28·25	25
	Aerated	41·25	22
25	Control	25·60	42
10	Anaerobic	21·10	31
	Control	25·55	53
	Aerated	30·40	66
26	Control	52·00	45
24A	Anaerobic	15·55	34
B	Anaerobic	24·95	39
C	Anaerobic	39·70	53
D	Anaerobic	55·40	83

On the whole, low values for precipitable nitrogen correspond to low bacterial numbers, and high values to high numbers. To test whether a direct mathematical relation between the two quantities does in fact exist, the regression of precipitable nitrogen on bacterial numbers was calculated.

If x = numbers of bacteria in 100 millions/ml. and y = precipitable nitrogen in mg./l., then

Mean value of $y = 39$, if x	$= 31·549$.
Regression coefficient of y on x	$= +0·9131$.
Estimated standard error of regression coefficient	$= 0·2984$.
t	$= 3·0601$.
Number of cases	$= 20$.

From a table of t (Fisher, 1936) it can be found that the regression coefficient differs significantly from zero; this shows that a direct linear relation between x and y does in fact exist.

This relation can be expressed by the equation

$$y = 10·183 + 0·913x.$$

Fig. 7 shows the calculated regression line, with the observed points, which are evenly distributed about it. It may reasonably be concluded that the greater amount of nitrogen remaining in aerated cultures after denitrification, and hence the smaller loss of nitrogen under aerobic conditions, is directly connected with the greater growth of the bacteria. The

denitrifying reaction was not always completed, as in several cases measurable amounts of "non-protein" nitrogen were left at the end of an experiment; but a state was approached in which, in the presence of an adequate amount of an available organic compound, only the nitrogen required for bacterial cell-substance was retained in the culture, and all the rest was lost as gas. This seems to be a much simpler and more satisfactory explanation of the smaller loss of nitrogen under aerobic conditions than the "diminished denitrifying power" postulated by earlier authors (cf. Seiser & Walz, 1925).

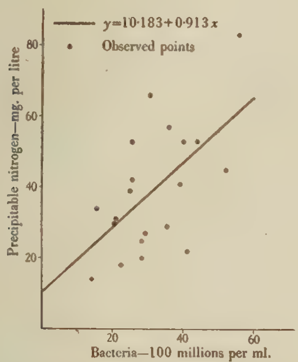

Fig. 7. Regression of precipitable nitrogen on bacterial numbers.

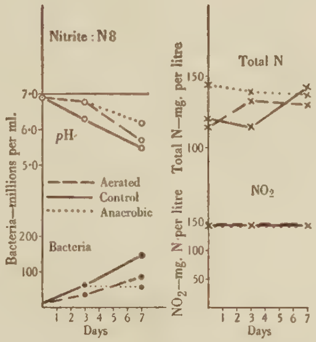

Fig. 8. Effect of nitrite on species N 8 in a neutral medium.

D. *The reduction of nitrite*

The reduction of nitrate by bacteria is divided into at least two stages: (1) the reduction of nitrate to nitrite, (2) the further reduction of nitrite. Nitrite is toxic to various organisms; yet in the normal course of denitrification the bacteria seem to be able to deal with fairly high concentrations of nitrite. It is, therefore, interesting to examine the effect on the bacteria if nitrate in the medium is replaced by nitrite. In the first experiment, all the nitrate in the medium was replaced by an equivalent amount of nitrite (140 mg. N/l.): the conditions of the experiment were: species N 8, air supply *all three levels*, *p*H 6·9, glucose 0·35 %, $NaNO_2$ 0·069 %, C/N ratio 10 (Fig. 8). In all three cultures, the nitrite reaction was as strong at the end of the experiment as at the beginning; in a neutral medium, nitrite added at the beginning is not used by the bacteria. There was very little growth, the highest count being 146 million cells/ml. in the control culture. There was no loss of total nitrogen (the apparent loss in the anaerobic culture is due to irregularities in the analysis, which was affected by nitrite). The final *p*H values were slightly acid in all three cases.

In order to find out whether the bacteria did not reduce nitrite because they could not use it for growth, or if it was actually poisonous to them, a second experiment was set up in which nitrate and nitrite were both present in the medium. The total nitrogen of the media was the same (139 mg. N/l.), but varying proportions of the nitrate were replaced by nitrite. Other conditions were: species N 8, air supply *control*, *p*H 6·9, glucose 0·35 %, C/N ratio 10 (see Table 6).

Table 6. *Effect of increasing doses of nitrite in neutral media*

Mg. N/l. of medium						
As NO_3	As NO_2	Days	Bacteria millions/ml.	*p*H	NO_2 mg. N/l.	Total N mg./l.
125	14	0	11	6·9	14	(139)
		14	3000	7·1	0	18
104	35	0	11	6·9	35	(139)
		14	1388	4·9	28	88
90	49	0	11	6·9	49	(139)
		14	848	4·9	<49	103
69	70	0	11	6·9	70	(139)
		14	332	4·7	70	111

These results show that nitrite was not reduced by species N 8 because it is toxic, and not merely because it cannot be used for growth. In all these media there is enough nitrate present to supply nitrogen for growth, but growth is diminished in all but the first medium. The toxic effects of nitrite increase with its concentration; species N 8 appears to tolerate nitrite equivalent to 14 mg. nitrogen/l., as at this concentration growth and denitrification are normal; but both processes are progressively affected by higher concentrations of nitrite in a neutral medium.

In an alkaline medium, however, the state of affairs is quite different. Karlsen (1938) pointed out that the optimum *p*H for the reduction of nitrite by *Ps. aeruginosa* is 8·1–8·6 for the highest concentration used; her strain also would not decompose nitrite in media more acid that *p*H 7·2. In consequence of Karlsen's results, the action of nitrite on species N 8 and 309 in alkaline media was investigated. Table 7 gives the results of a double experiment in media with initial *p*H 6·9 and 8·0: other conditions were: species 309, air supply *anaerobic*, glycerol 0·358 %, C/N ratio 10.

Table 7. *Effect of increasing doses of nitrite on* 309

Mg. N/l. of medium			Neutral			Alkaline		
As NO_3	As NO_2	Days	Bacteria millions/ml.	*p*H	NO_2 mg. N/l.	Bacteria millions/ml.	*p*H	NO_2 mg. N/l.
139	0	0	10	6·9	0	10	8·0	0
		7	2850	7·3	0	2695	7·2	0
104	35	0	10	6·9	35	10	8·0	35
		7	2100	7·4	0	2320	7·3	0
69	70	0	10	6·9	70	10	8·0	70
		7	280	6·7	70	1820	7·3	0
0	140	0	10	6·9	140	10	8·0	140
		7	26	6·6	140	1760	8·0	0

The total nitrogen estimations were seriously affected by the presence of nitrite, and are not included in Table 7; but the figures in the nitrite column show whether it was reduced or not. In the neutral medium, the higher concentrations of nitrite are progressively toxic to species 309, and are not reduced. But in the alkaline medium, species 309 will grow normally and denitrify on all the concentrations of nitrite given, even where nitrite is the only source of nitrogen in the medium. At the highest concentration of nitrite the number of bacteria is slightly diminished, but this is the only indication of any toxic action at all.

Species N 8 also is not poisoned by nitrite in alkaline solution; the results of an experiment in which nitrate alone was compared against nitrite alone are given in Table 8: all the media contained about 140 mg. nitrogen/l. and had a C/N ratio of 10.

Table 8. *Nitrite in alkaline solution—species* N 8

Air-supply	Medium: Reaction	Medium: Nitrogen source	Medium: Carbon source	Days	Bacteria millions/ml.	pH	NO_2 mg. N/l.	Total N mg. N/l.
Control	Alkaline	NO_3	Glycerol	0	11	8·0	0	114
				7	1700	8·4	0	32
Control	Alkaline	NO_2	Glycerol	0	11	8·0	140	119
				7	3020	8·4	0	34
Control	Alkaline	NO_2	Glucose	0	11	8·0	140	109
				7	424	5·5	c. 100	74
Control	Neutral	NO_2	Glycerol	0	11	6·9	140	123
				7	48	6·9	140	119
Anaerobic	Alkaline	NO_3	Glycerol	0	12	8·0	0	151
				14	2415	8·4	0	90
Anaerobic	Alkaline	NO_2	Glycerol	0	12	8·0	140	(Lost)
				14	1640	8·4	0	65

The results included in Table 8 can be summed up as follows: (1) in a neutral medium nitrite is poisonous to species N 8 in presence of glycerol. (2) N 8 will grow normally and reduce nitrate in an alkaline glycerol medium under both conditions of air supply. (3) The toxic action of nitrite is cancelled in alkaline solution in presence of glycerol. (4) If glucose is used as the substrate, nitrite still appears to be poisonous in an initially alkaline medium; the cause of this is probably the acid formed in the breakdown of glucose.

E. *Utilization of the carbon source*

All the preceding experiments have been mainly concerned with the nitrogen content of the cultures; it has been briefly indicated that nitrate reduction is affected by variations in the C/N ratio, but the effect on the carbon-source itself has not been considered. Glucose was used as substrate in a series of experiments at different C/N ratios, and its presence or absence determined, at the end of incubation, by Fehling's or Cole's test. The results obtained show an interesting difference between aerobic and anaerobic cultures of species N 8.

In the first experiment, the C/N ratio of the medium was 10 (glucose 0·35 %, KNO_3 0·1 %, pH 7·1). Two control cultures of species N 8 were compared against two anaerobic cultures.

Table 9. *Glucose left at* C/N *ratio* 10

Air-supply	Days	Bacteria millions/ml.	pH	NO_2 mg.N/l.	Total N mg.N/l.	Glucose at end of experiment
Control A	0	7	7·1	0	142	Absent
	14	2560	8·1	0	58	
Control B	0	7	7·1	0	142	Absent
	14	4900	7·9	0	47	
Anaerobic A	0	7	7·1	0	142	Present
	14	1490	8·5	0	75	
Anaerobic B	0	7	7·1	0	142	Present
	14	1520	9·0	0	43	

In all four cultures denitrification proceeded normally; and in the aerobic cultures all the glucose was used. But in the anaerobic cultures not all the glucose was used, and the numbers of bacteria were smaller. It seems, therefore, that at a C/N ratio of 10, enough

nitrate is present in the aerobic cultures for full growth and decomposition of the glucose; but in the anaerobic cultures both processes are limited by the amount of nitrate available.

A second experiment at C/N ratio 5, with twice the amount of nitrate, gave similar results (glucose 0·35 %, KNO_3 0·2 %, *p*H 6·8, species N 8).

Table 10. *Glucose left at* C/N *ratio* 5

Air-supply	Days	Bacteria millions/ml.	*p*H	NO_2 mg. N/l.	Total N mg. N/l.	Non-protein mg. N/l.	"Protein" mg. N/l.	Glucose at end of experiment
Aerated	0	10	6·8	0	235	—	—	—
	7	3040	8·6	0	66	0	66	
Control	0	10	6·8	0	237	—	—	
	7	2470	8·2	0	58	13	45	Absent
	14	2555	8·5	0	53	0	53	
Anaerobic	0	10	6·8	0	222	—	—	Present
	7	1800	8·2	0	33	9	24	
	14	2110	8·2	0	31	0	31	

Dentrification is complete in all three cultures, as is shown by the disappearance of "non-protein" nitrogen, but again at this C/N ratio there is not sufficient nitrate present in the anaerobic culture for the glucose to be completely used. The difference in numbers of bacteria is not quite so great as at C/N ratio 10. If the C/N ratio is narrowed still more by increasing the amount of nitrate with the same amount of glucose, no very striking changes are produced in cultures of N 8 under aerobic (control) conditions. An experiment on these lines showed abundant growth, alkaline drift in *p*H, loss of total nitrogen, and complete utilization of the glucose, at nitrate concentrations of 0·4 and 0·8 %; at the latter concentration (1109 mg. N/l., C/N = 1·25) there was a small amount of nitrite left in the medium after 15 days. The number of bacteria present at this concentration of nitrate was slightly smaller than the number in the parallel culture with 0·1 % nitrate. Under anaerobic conditions, on the other hand, the narrowing of the C/N ratio produces striking changes. The results of such an experiment with strain N 8 are given in Table 11.

Table 11. *Effect of* C/N *ratio in anaerobic cultures*

Conc. KNO_3 %	C/N ratio	Days	Bacteria millions/ml.	*p*H	NO_2 mg. N/l.	Total N mg. N/l.	Non-protein mg. N/l.	"Protein" mg. N/l.	Glucose at end of experiment
0·1	10	0	11	6·8	0	122	—	—	Present
		15	1555	8·6	0	60	26	34	
0·2	5	0	11	6·8	0	(278)	—	—	Present
		15	2495	8·8	0	72	33	39	
0·4	2·5	0	11	6·8	0	(554)	—	—	Absent
		15	3970	9·0	0	81	28	53	
0·8	1·25	0	11	6·8	0	(1109)	—	—	Absent
		15	5540	8·8	60	259	176	83	

The numbers of bacteria increase progressively as the nitrate concentration increases, from a value characteristic of those previously observed in anaerobic cultures at C/N 10, to a value as large as any observed in aerated cultures. It therefore appears probable that the differences in bacterial numbers between aerobic and anaerobic cultures at C/N 10 are caused by the limiting effect of the amount of nitrate available under anaerobic conditions. The amount of precipitable nitrogen increases with the nitrate concentration, roughly in

proportion with the bacterial numbers. At C/N ratios 10 and 5 there is not enough nitrate present for complete utilization of the glucose, and some is left at the end of incubation. At C/N ratio 2·5 the supply of nitrate is sufficient for the glucose to be completely used; and at C/N 1·25 there is a little too much nitrate, as is shown by the presence of nitrite at the end of the experiment, and by the large figure obtained for non-protein nitrogen, in contrast to the small and approximately constant quantity recorded in the other three cultures. Strain N 8, therefore, when grown in anaerobic conditions, requires more nitrate, relative to the amount of organic matter supplied, than it does in the presence of air.

Discussion

Species N 8 and 309 have simple requirements for growth; both species can grow on a synthetic medium with nitrate as the only source of nitrogen, and the limiting *p*H values for species 309—3·9 and 9·5—are wide apart. A variety of organic compounds could be used as substrates for growth, but not all of them acted as hydrogen-donators for denitrification; failure of denitrification usually involved the later stages of the reaction only; the nitrate was reduced to nitrite, but no further.

The results of the experiments on aeration show that nitrogen is lost from cultures at all three levels of air supply. This loss is accompanied by the same changes in the culture that were recorded as signs of apparent denitrification in the first experiments; they include an alkaline drift in *p*H (preceded by slight acid formation in glucose cultures). As Weissenberg (1902) pointed out, true denitrification is always accompanied by an increase in alkalinity of the medium; and the *p*H values observed exclude the possibility of so-called "indirect" denitrification, i.e. the formation of nitrogen in the reaction between nitrite and ammonia or amines, since this reaction only takes place in acid solution. The cultures in several experiments were tested for ammonia with Nessler's reagent, but none was found. Nitrogen was always lost most rapidly in the first few days of the incubation period, and the reduction was complete after about 10 days' incubation at room temperature.

The amount of nitrogen remaining after the reduction of the nitrate was highest in aerated, and lowest in anaerobic, cultures; and most of it was "protein" nitrogen. Von Caron (1912) recorded that more protein was formed in aerated liquid cultures of three denitrifiers than in anaerobic cultures, and Seiser & Walz (1925) compared anaerobic and undisturbed aerobic cultures of *Ps. putida*, and found that consistently more nitrogen was retained in aerobic cultures. They associated this with increased bacterial growth; but they made no counts of cell numbers, being content with eye estimates of turbidity. In the present case, as counts showed that the numbers of bacteria increased with increased aeration, and as "protein" nitrogen was found to be directly proportional to bacterial numbers, the greater amount of nitrogen retained in aerated cultures can be attributed to greater growth of the bacteria. Less nitrogen is lost from aerated than from anaerobic cultures, not because the bacteria have a greater "denitrifying power" under anaerobic conditions, but because the larger numbers of bacteria in aerated cultures lock up more nitrogen in their cell proteins.

Karlsen (1938) showed that nitrite was toxic to *Ps. aeruginosa* in neutral or faintly acid solution, but was harmless and freely reduced to nitrogen in alkaline solution (optimum *p*H 8·1–8·6). Strains 309 and N 8 are also poisoned by nitrite at *p*H 6·9, even in the presence of nitrate, but freely reduce it at *p*H 8·0. The poisonous effect of nitrite at *p*H 6·9 is pro-

portional to the concentration; this fact, as Karlsen pointed out, invalidates the explanation given by Elema (1932) for the toxicity of nitrite. He attributed it to the high oxidation-reduction potential produced by the presence of nitrite in acid solutions. But, as Elema himself shows, the potential level in denitrifying cultures is dependent on the presence, and not on the concentration, of nitrite. It is possible, as Karlsen suggests, that free undissociated nitrous acid is considerably more poisonous than the nitrite ion.

The changes produced in anaerobic cultures of species N 8 by varying the C/N ratio make it evident that, when grown anaerobically, this species requires more nitrate, relative to the amount of energy source, than it does under aerobic conditions. This is explicable on the hypothesis that nitrate is used as a source of oxygen as well as a source of nitrogen by the organism growing anaerobically. In other words, anaerobic denitrification can be explained by the "oxygen need" theory. But this theory will not account for the fact of denitrification under aerobic conditions. When air is bubbled through the culture, the bacteria will still completely reduce all the nitrate present, and retain only the nitrogen used to build up their cell proteins. The enzyme system concerned in denitrification does not appear to be the same as the *nitratase* of Quastel *et al.* (1925), by means of which *Bact. coli* reduces nitrate to nitrite, and is enabled to grow anaerobically in presence of nitrate and a hydrogen-donator. For, as Stickland (1931) showed, the nitratase system of *Bact. coli* is inhibited by oxygen; a gas mixture containing 20·9 % oxygen (≡air) causes a 94 % inhibition, and the enzyme is perceptibly inhibited by very small amounts of oxygen (down to 0·36 %). It may, therefore, be said that aerobic denitrification undoubtedly takes place, but cannot be satisfactorily explained on any existing theory.

Summary

1. Two species of *Pseudomonas* are described, which reduce nitrate to nitrite and nitrogen gas in simple synthetic media.

2. An adequate supply of a suitable organic compound is necessary for denitrification.

3. Both species will denitrify in aerated, and in undisturbed aerobic cultures, as well as under anaerobic conditions.

4. At C/N ratio 10, the bacteria grow to higher numbers in aerobic than in anaerobic cultures. The amount of precipitable nitrogen retained in a culture is directly proportional to the bacterial numbers, and therefore the smaller loss of nitrogen from aerobic compared with anaerobic cultures is a consequence of the greater growth of the bacteria.

5. At pH 6·9 nitrite has a poisonous effect, proportional to its concentration, on both species; but at pH 8·0 it is harmless and freely reduced.

6. Species N 8 requires more nitrate, relative to the amount of organic matter present, under anaerobic than under aerobic conditions.

REFERENCES

von Caron, H. (1912). Untersuchungen über die Physiologie denitrifizierender Bakterien. *Zbl. Bakt.* Abt. II, **33**, 62.

Dowson, W. J. (1939). On the systematic position and generic names of the Gram-negative bacterial plant pathogens. *Zbl. Bakt.* Abt. II, **100**, 177.

Elema, B. (1932). Bepaling van de Oxydatie-Reductiepotentiaal in Bacteriëncultures en hare Beteeknis voor de Stofwisseling. *Proefschrift*, Delft.

ELEMA, B., KLUYVER, A. J. & VAN DALFSEN, J. W. (1934). Stoffwechselvorgangen und Oxydoreduktionspotential. I. Versuche mit denitrifizierenden Bakterien. *Biochem. Z.* **270**, 317.

FISHER, R. A. (1936). *Statistical Methods for Research Workers*, 6th ed. Edinburgh: Oliver and Boyd.

KARLSEN, A. (1938). Denitrification and hydrogen-ion concentration. *Bergens Mus. Aarb.* Nr. 2.

KLAESER, M. (1914). Die Reduktion von Nitraten zu Nitriten und Ammoniak durch Bakterien. *Zbl. Bakt.* Abt. II, **41**, 365.

KOROCHKINA, O. I. (1936). The oxidation-reduction regime of denitrification. *Microbiology*, **5**, 645. (Russian, English summary.)

MEIKLEJOHN, J. (1937). The oxygen uptake of suspensions and cultures of a free-living bacterium. *J. exp. Biol.* **14**, 158.

PUCHER, G. W., LEAVENWORTH, C. S. & VICKERY, H. B. (1930). Determination of total nitrogen of plant extracts in presence of nitrates. *Industr. Engng Chem.* Anal. ed. **2**, 191.

QUASTEL, J. H., STEPHENSON, M. & WHETHAM, M. D. (1925). Some reactions of resting bacteria in relation to anaerobic growth. *Biochem. J.* **19**, 304.

SEISER, A. & WALZ, L. (1925). Stickstoffumsatz bei der Denitrifikation. *Arch. Hyg., Berl.*, **95**, 189.

STICKLAND, L. H. (1931). The reduction of nitrates by *Bact. coli*. *Biochem. J.* **25**, 1543.

STILES, H. R., PETERSON, W. H. & FRED, E. B. (1926). A rapid method for the determination of sugar in bacterial cultures. *J. Bact.* **12**, 427.

WEISSENBERG, H. (1902). Über die Denitrifikation. *Zbl. Bakt.* Abt. II, **8**, 166.

WOOLF, B. (1928). The estimation of ammonia, urea, and total nitrogen. *Chem. Ind. Rev.* **6**, 1060 (summary).

ZoBELL, C. E. (1932). Factors influencing the reduction of nitrates and nitrites by bacteria in semisolid media. *J. Bact.* **24**, 273.

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REVIEWS

The Fundamentals of Fruit Production. By V. R. Gardner, F. C. Bradford and H. D. Hooker. 2nd edition. Pp. xvi + 788. New York and London: McGraw-Hill Book Co. 1939. 30*s.* 0*d.*

At the time of its publication in 1922 the first edition of this book was a splendid survey of the physiological data and principles underlying fruit production, as these could then be formulated. During the intervening period, however, new and revolutionary concepts of widespread implication have been put forward, and many primary data have received radically different interpretation: unfortunately little of this shows in the present work. True, new data have been incorporated into every chapter of the second edition, which has been increased in length by 102 pages, but these new data are only such as fit into the detailed scheme crystallized in the 1922 volume, and the authors' viewpoints have, in all essentials, remained unchanged. The book is still an interesting and valuable survey, especially in those fields where the newer outlook has not changed the whole perspective, but, generally speaking, the second edition is nothing like so good a book for 1939 as its predecessor was for 1922.

W. B. BRIERLEY

Herbage Publication Series. Imperial Bureau of Pastures and Forage Crops, Aberystwyth.

Bull. 26. *Research on Grassland, Forage Crops and the Conservation of Vegetation in the United States of America.* Compiled by R. O. Whyte. Pp. 113. Sept. 1939. Board 5*s.* 0*d.*

Bull. 27. *The Control of Weeds.* Edited by R. O. Whyte. Pp. 168. Jan. 1940. Board 7*s.* 6*d.*

Bull. 28. *Technique of Grassland Experimentation in Scandinavia and Finland.* Pp. 52. Jan. 1940. Paper 2*s.* 6*d.*

Bull. 29. *Grassland Investigations in Australia.* Pp. 107. Jan. 1940. Board 5*s.* 0*d.*

Bull. 26. An excellent and suggestive summary of current investigations of problems relating to the improvement of pastures and range lands, the production of forage crops, and the utilization of grasses and other vegetation in the conservation of soil in the United States, by various Government research services, the U.S. Golf Association, the Division of Plant Biology of the Carnegie Institute of Washington, and 46 State agricultural experiment stations. There are maps and illustrations and subject and plant indexes. The bulletin will interest not only agriculturists and agricultural botanists, but plant pathologists, applied entomologists, and geneticists.

Bull. 27. A valuable symposium by 11 specialists from Canada, the United States, Germany, Australia, New Zealand, and South Africa on the importance of weeds, legislative measures, cultural, chemical, and biological methods of control, and poisonous weeds. There are useful bibliographies, numerous good illustrations, and subject and genera indexes.

Bull. 28. A description by 7 Finnish, Danish, Swedish, and Norwegian workers of their methods of grassland and pasture experimentation, of botanical and chemical grassland analyses, and of experiments with strains and seeds mixtures. There are two maps showing the location of agricultural research stations in the four countries.

Bull. 29. A brief account of Institutions and Departments engaged in pasture work followed by 7 special articles on ecological aspects, plant improvement, natural pastures, weeds, and insect pests, and 8 articles on current research in various regions. There are 247 abstracts of recent publications on grassland in Australia, an index of authors in the abstract section, and an index of genera.

May one suggest to the Aberystwyth Bureau that if abbreviated titles, bulletin numbers, and year of publication, were printed on the spine of suitable issues it would save much time and exasperation on the part of those wishing to consult their publications.

W. B. BRIERLEY

The Scientific Principles of Plant Protection with Special Reference to Chemical Control. By HUBERT MARTIN. 3rd edition. Pp. x+385. London: Edward Arnold & Co. 1940. 22*s*. 6*d*.

Dr Martin states: "The present edition therefore follows the plan of the second edition but, in order to include a discussion of the many developments of the subject within the past four years, a drastic pruning of the text was necessary. The book has been reset for this, the third edition. The subjects in which the greatest advance has been made include the nature and control of virus diseases, the epidemiological factors determining the degree of attack and the interpretation, to practical conditions, of the results of the laboratory assessment of the qualities which determine insecticidal and fungicidal efficiency. With knowledge of the reasons for the efficiency of the old-established but empirically-discovered insecticides and fungicides, it has become possible to compound both new and old toxic products to simple, fool-proof pest control materials. The end of the home-made insecticide and fungicide and its replacement by the compounded product is in sight."

Dr Martin is fortunate in that at Long Ashton he can obtain criticism and help from such able colleagues but, even so, in view of the very rapid development and the ever widening scope of the subject of Plant Protection (in this edition the author index runs to 1171 names) he might, perhaps, find it advisable to seek further assistance in the preparation of subsequent editions. For example: in his consideration of "Weed Killers" (ch. x) it is quite clear that Dr Martin is writing with little first-hand knowledge of the subject. He makes no reference to the very important work carried out during the last decade by the "Associate Committee on Weed Control" of the National Research Council of Canada, practically no reference to the important work in the United States during the same period, and no reference to the numerous and important researches carried out in various European countries, notably Germany, during the last 30 years. Quite bluntly, the chapter is both parochial and out-of-date and, unfortunately, this chapter does not stand alone in its need of drastic emendation.

In spite, however, of being open to criticism, this third edition of *The Scientific Principles of Plant Protection* is a brilliant achievement and is the only treatise of its kind in the English language. All teachers, advisory and research workers, whose concern is healthy plants, will find it just as indispensable as were the earlier editions. W. B. BRIERLEY

Handbook of Phytopathogenic Viruses. By F. O. HOLMES. Pp. 221. Minneapolis, Minn. U.S.A.: Burgess Publishing Co. 1940. $2·00.

This book by one of the foremost American workers on plant viruses presents a courageous attempt to name and classify these agents. Dr Holmes proposes an extension of the binomial system to include the viruses and he has shown great ingenuity in the selection of so many Latin names, *Marmor dubium* for potato virus X is a case in point. While, admittedly, the binomial system has many advantages it is doubtful if it can be applied successfully to such things as viruses. To the reviewer the chief drawback to this attempt at classification is the emphasis laid on symptoms. The whole classification is in consequence just as arbitrary as the much criticized system now in general use because a virus is classified by the symptoms it produces on one particular host. By this reasoning one virus has equal claims to several places in the classification according to which plant it is infecting. The largest family is the Marmoraceae or mosaic group and this is a heterogeneous collection of viruses. For example, why include tobacco necrosis virus in the mosaic group? It has never been shown to cause a mottling in any host plant. It would surely be better in the family Lethaceae, genus *Lethum*, since it causes only death of the cells, but that family is reserved for thrips-transmitted viruses of the spotted wilt type. Indeed, so many different viruses seem to be included in the Marmoraceae that a system of classification of these is urgently required. Another criticism is the erection of only one genus in each family, which seems to make the former both unwieldy and redundant.

Apart from the classification, the book contains, in concise form, much valuable information about each virus. The book itself is of a compact loose-leaf type and its format will be of interest to English readers since it is a photographic reproduction of typescript. K. M. SMITH

Plant Viruses and Virus Diseases. By F. C. BAWDEN. Pp. 272. Leiden: Chronica Botanica Co. (London: Wm. Dawson & Sons). 1939. Guilders 7.

Until less than a decade ago plant and animal viruses and virus diseases formed somewhat isolated and obscure territories within the boundaries of plant and animal pathology with bacteriology and physiology as their nearest, albeit rather suspicious, neighbours. Then, five years ago came Stanley's discovery which, almost overnight, threw these preserves wide open. Physicists, chemists, crystallographers, and many other workers flocked in and so rapid was the development that, in Mr Bawden's opinion, "the necessity for separating viruses from other recognized pathogens is becoming more obvious, and their adoption by protein chemists seems only a matter of time". True, it has now been shown with reasonable certainty that some plant viruses are nucleoproteins, and Mr Bawden may be right in his conjecture. On the other hand, there is a great deal of work on animal viruses which is difficult to align with this view, and many of us have a rather uneasy feeling that viruses may be of different natures having only their relative size and disease-causing ability in common.

Furthermore, a virus is not sufficient unto itself, it is only a part of a total complex which is a diseased host. Even if the protein chemist adopts the virus *qua* virus it still remains for the pathologist to study the disease *qua* disease. As Mr Bawden admits on his last page, "although our knowledge on the nature of viruses and their properties *in vitro* has greatly increased in recent years, we are still almost entirely ignorant of their activities *in vivo*". The study of all aspects of diseased plants as living hosts, of disease epidemiology, of agricultural, economic, and other environmental relationships; these are concrete problems for the pathologist. The study of virus diseases of plants is infinitely greater than the study of the mere viruses, and I sometimes wonder whether no little delay and confusion is not produced by their frequent equation. It is the kind of situation with which we have long been familiar in the equation of mycology with plant pathology: virologists no more than mycologists are necessarily plant pathologists, and if protein chemists take charge they will become even less so.

However, Mr Bawden does not intend his book to be a textbook of plant virus diseases and claims only that "It is an attempt to describe and correlate the advances that have been made recently in the study of plant viruses". The only aspects he specifically excludes are "detailed descriptions of symptoms and host ranges", but the claim is not entirely justified that "all other aspects of the subject are treated". The chapter headings show the general scope of the volume: (1) Introductory survey; (2) Symptomatology—external symptoms; (3) Symptomatology—internal symptoms; (4) Transmission and properties in expressed sap; (5) Mechanism of insect-transmission and relationships between viruses and their vectors; (6) Virus strains, mutation, and acquired immunity; (7) Serological reactions of plant viruses; (8) Purification of viruses; (9) Properties of purified virus preparations; (10) Optical properties of purified virus preparations; (11) The sizes of viruses; (12) Correlation of virus activity with the isolated nucleoproteins; (13) Physiology of virus-diseased plants; (14) Classification and control; (15) Discussion on the origin and multiplication of viruses. Bibliographies which are not entirely consistent terminate the chapters; there are 37 text illustrations, and author and subject indexes.

What Mr Bawden has really done is to write a book for the handful of people like himself, throwing parts of chapters 2, 3, 13 and 14 as sops to plant pathologists, and chapter 15 as a sop to theoretically minded general biologists. But for anyone wishing to know what is being done in this restricted field and why it is being done, I know of no book so helpful. Incidentally, chapter 6 seems to me below the level of the rest of the book, whilst the author's views on classification seem more destructive than constructive. The author writes not always grammatically but with unusual vigour and freshness of outlook, and presents a remarkably clear and stimulating picture of what is really an obscure and rapidly changing situation. One obvious general criticism is that Mr Bawden's vision is just a little myopic so that he sees his own potato patch as the most important feature of the landscape. For example, the author index contains the names of 242 workers, of which only three receive more than twenty-five page references; Bawden heads the list with thirty-nine, Pirie follows with thirty-six, and Stanley comes in a bad third with only twenty-seven; many well-known virus names do not appear at all. Little things like this, however, give the book a very personal quality which receives emphasis by the inclusion, here and there, of unpublished work by the author or his collaborators.

Whatever sins of omission or commission this book contains they are largely due to the fine enthusiasm and abounding energy of its author. Mr Bawden has views about most virus problems and he does not hesitate to state them vigorously and to carry his attack into opposing camps. In this book he has achieved a fine task and his book is one of the most useful additions of recent years to plant virus literature.

W. B. BRIERLEY